

Requested Patent: WO0214281A1

Title: PYRIDINE DERIVATIVES AS INHIBITORS OF P38 ;

Abstracted Patent: WO0214281 ;

Publication Date: 2002-02-21 ;

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Application Number: WO2001US25015 20010810 ;

Priority Number(s): US20000224719P 20000811 ;

IPC Classification:

C07D213/75; C07D401/12; A61K31/4418; A61P7/00; A61P9/00; A61P17/00;
A61P25/00; A61P31/00; C07F9/58 ;

Equivalents: AU8323701, CA2418389, EP1309560, JP2004506042T ;

ABSTRACT:

The present invention relates to inhibitors of p38, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli. The invention also relates to inhibitors of ZAP70. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders. The compounds have general formula: (Ia), (Ib), (Ic) and (Id).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 February 2002 (21.02.2002)

PCT

(10) International Publication Number
WO 02/14281 A1

(51) International Patent Classification⁷: C07D 213/75,
401/12, A61K 31/4418, A61P 7/00, 9/00, 17/00, 25/00,
31/00, C07F 9/58

(21) International Application Number: PCT/US01/25015

(22) International Filing Date: 10 August 2001 (10.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/224,719 11 August 2000 (11.08.2000) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 02/14281 A1

(54) Title: INHIBITORS OF p38

(57) Abstract: The present invention relates to inhibitors of p38, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli. The invention also relates to inhibitors of ZAP70. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

PYRIDINE DERIVATIVES AS INHIBITORS OF P38

TECHNICAL FIELD OF INVENTION

5

The present invention relates to inhibitors of p38, a mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for
10 producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

BACKGROUND OF THE INVENTION

15

Protein kinases are involved in various cellular responses to extracellular signals. Recently, a family of mitogen-activated protein kinases (MAPK) has been discovered. Members of this family are Ser/Thr kinases that activate their substrates by phosphorylation
20 [B. Stein et al., Ann. Rep. Med. Chem., 31, pp. 289-98 (1996)]. MAPKs are themselves activated by a variety of signals including growth factors, cytokines, UV radiation, and stress-inducing agents.

One particularly interesting MAPK is p38. p38,
25 also known as cytokine suppressive anti-inflammatory drug binding protein (CSBP) and RK, was isolated from murine pre-B cells that were transfected with the lipopolysaccharide (LPS) receptor, CD14, and induced with LPS. p38 has since been isolated and sequenced, as has
30 the cDNA encoding it in humans and mouse. Activation of

p38 has been observed in cells stimulated by stress, such as treatment of lipopolysaccharides (LPS), UV, anisomycin, or osmotic shock, and by cytokines, such as IL-1 and TNF.

5 Inhibition of p38 kinase leads to a blockade on the production of both IL-1 and TNF. IL-1 and TNF stimulate the production of other proinflammatory cytokines such as IL-6 and IL-8 and have been implicated in acute and chronic inflammatory diseases and in post-
10 menopausal osteoporosis [R. B. Kimble et al., Endocrinol., 136, pp. 3054-61 (1995)].

 Based upon this finding, it is believed that p38, along with other MAPKs, have a role in mediating cellular response to inflammatory stimuli, such as
15 leukocyte accumulation, macrophage/monocyte activation, tissue resorption, fever, acute phase responses and neutrophilia. In addition, MAPKs, such as p38, have been implicated in cancer, thrombin-induced platelet
20 aggregation, immunodeficiency disorders, autoimmune diseases, cell death, allergies, osteoporosis and neurodegenerative disorders. Inhibitors of p38 have also been implicated in the area of pain management through inhibition of prostaglandin endoperoxide synthase-2
25 induction. Other diseases associated with IL-1, IL-6, IL-8 or TNF overproduction are set forth in WO 96/21654.

 Others have already begun trying to develop drugs that specifically inhibit MAPKs. For example, PCT publication WO 95/31451 describes pyrazole compounds that inhibit MAPKs, and, in particular, p38. However, the
30 efficacy of these inhibitors *in vivo* is still being investigated.

Other p38 inhibitors have been produced, including those described in WO 98/27098, WO 99/00357, WO 99/10291, WO 99/58502, WO 99/64400, WO 00/17175 and WO 00/17204.

5 Accordingly, there is still a great need to develop other potent inhibitors of p38, including p38-specific inhibitors, that are useful in treating various conditions associated with p38 activation.

 Another protein kinase that is involved in
10 cellular responses to extracellular signals is ZAP70. When the T cell receptor (TCR) in T cells is triggered by binding an antigen, it in turn activates ZAP70. ZAP70 acts to couple the TCR to a number of essential signalling pathways that are required for T cell
15 differentiation and proliferation.

 Given ZAP70's role in T cell signalling, ZAP70 may have a role in T cell mediated diseases. Such diseases include, without limitation, transplantation, autoimmune disease, e.g., RA, systemic lupus erythematosus
20 (SLE), psoriasis, Sjogren's Syndrome, thyroiditis, pulmonary fibrosis, bronchiolitis obliterans, hemolytic anemia and Wegener's granulomatosis, cancer, including leukemia and lymphoma, multiple sclerosis, graft versus host disease, and Kawasaki syndrome.

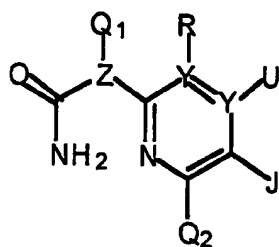
25 Accordingly, there is a great need to develop inhibitors of ZAP70 that are useful in treating various conditions associated with ZAP70 activation.

SUMMARY OF THE INVENTION

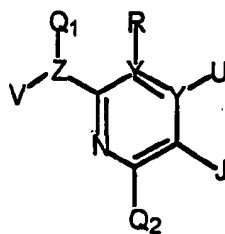
30 The present invention addresses this problem by providing compounds that demonstrate inhibition of p38 and/or ZAP70.

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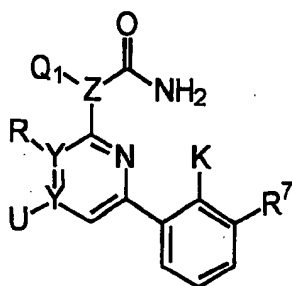
These compounds have the general formula:



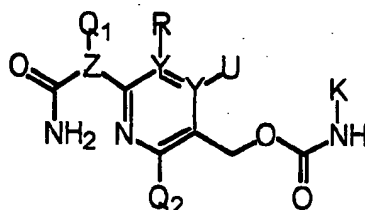
(Ia),



(Ib),



(Ic)



and (Id),

wherein each of Q_1 and Q_2 are independently selected from a phenyl or 5-6 membered aromatic heterocyclic ring system, or a 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring.

A heterocyclic ring system or a heterocyclic ring contains 1 to 4 heteroatoms, which are independently selected from N, O, S, SO and SO_2 .

The rings that make up Q_1 are substituted with 1 to 4 substituents, each of which is independently selected from halo; C_1 - C_3 alkyl optionally substituted with NR'_2 , OR' , CO_2R' or $CONR'_2$; O-(C_1 - C_3)-alkyl optionally substituted with NR'_2 , OR' , CO_2R' or $CONR'_2$; NR'_2 ; OCF_3 ; CF_3 ; NO_2 ; CO_2R' ; $CONR'$; SR' ; $S(O_2)N(R')_2$; SCF_3 ; CN ; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$;

$N(R')S(O_2)R^4$; $N(R')R^4$; $N(R^4)_2$; OR^4 ; $OC(O)R^4$; $OP(O)_3H_2$; or $N=C-N(R')_2$.

The rings that make up Q_2 are optionally substituted with up to 4 substituents, each of which is independently selected from halogen; C_1-C_3 straight or branched alkyl optionally substituted with R' , NR'_2 , OR' , CO_2R' , $S(O_2)N(R')_2$, $N=C-N(R')_2$, R^3 , $O-P(O_3)H_2$, or $CONR'_2$; $O-(C_1-C_3)$ -alkyl; $O-(C_1-C_3)$ -alkyl optionally substituted with NR'_2 , OR' , CO_2R' , $S(O_2)N(R')_2$, $N=CR'-N(R')_2$, R^3 , $OP(O_3)H_2$, or $CONR'_2$; NR'_2 ; OCF_3 ; CF_3 ; NO_2 ; CO_2R' ; $CONR'_2$; R^3 ; OR^3 ; NR^3_2 ; SR^3 ; $C(O)R^3$; $C(O)N(R')R^3$; $C(O)OR^3$; SR' ; $S(O_2)N(R')_2$; SCF_3 ; $N=CR'-N(R')_2$; OR^4 ; $O-CO_2R^4$; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$; $N(R')S(O_2)R^4$; $N(R')R^4$; $N(R^4)_2$; OR^4 ; $OC(O)R^4$; $OP(O)_3H_2$; K ; or CN .

Each R' is independently selected from hydrogen; (C_1-C_3) -alkyl; (C_2-C_3) -alkenyl or alkynyl; phenyl or phenyl substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl; or a 5-6 membered heterocyclic ring system optionally substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl.

Each R is independently selected from hydrogen, $-R^2$, $-N(R^2)_2$, $-OR^2$, SR^2 , $-C(O)-N(R^2)_2$, $-S(O_2)-N(R^2)_2$, $-C(O)-OR^2$ or $-C(O)R^2$ wherein two adjacent R are optionally bound to one another and, together with each Y to which they are respectively bound, form a 4-8 membered carbocyclic or heterocyclic ring.

Each R^2 is independently selected from hydrogen; or (C_1-C_3) -alkyl or (C_1-C_3) -alkenyl, each

optionally substituted with $-N(R')_2$, $-OR'$, SR' , $-O-C(O)-N(R')_2$, $-C(O)-N(R')_2$, $-S(O_2)-N(R')_2$, $-C(O)-OR'$, $-NSO_2R^4$, $-NSO_2R^3$, $-C(O)N(R')(R^3)$, $-NC(O)R^4$, $-N(R')(R^3)$, $-N(R')(R^4)$, $-C(O)R^3$, $-C(O)N(R')(R^4)$, $-N(R^4)_2$, $-C(O)N=C(NH)_2$ or R^3 .

- 5 Each R^3 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or heterocyclic ring systems each optionally substituted with R' , R^4 , $-C(O)R'$, $-C(O)R^4$, $-C(O)OR^4$ or $-K$; or an 8-10 membered bicyclic ring system comprising aromatic
- 10 carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R' , R^4 , $-C(O)R'$, $-C(O)R^4$, $-C(O)OR^4$ or $-K$.

- Each R^4 is independently selected from R' ; (C_1-C_7) -straight or branched alkyl optionally substituted
- 15 with R' , $N(R')_2$, OR' , CO_2R' , $CON(R')_2$, $SO_2N(R')_2$ or $SO_2N(R^5)_2$; or a 5-6 membered carbocyclic or heterocyclic ring system optionally substituted with $N(R')_2$, OR' , CO_2R' , $CON(R')_2$, $SO_2N(R')_2$ or $SO_2N(R^5)_2$.

- 20 Each R^5 is independently selected from hydrogen, (C_1-C_3) -alkyl, or (C_1-C_3) -alkenyl; each optionally substituted with $-N(R')_2$, $-OR'$, SR' , $-C(O)-N(R')_2$, $-S(O_2)-N(R')_2$, $-C(O)-OR'$, $-N-S(O_2)(R')$, $-NSO_2R^6$, $-C(O)N(R')(R^6)$, $-NC(O)R'$, $-N(R')(R^6)$, $-C(O)R^6$,
- 25 $-C(O)N=C(NH)_2$ or R^6 .

 Each R^6 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or heterocyclic ring systems each optionally substituted with R' , $-C(O)R'$ or $-C(O)OR'$; or an 8-10 membered

bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R', -C(O)R' or
5 C(O)OR'.

R⁷ is selected from H, halogen, or a (C₁-C₃) straight chain or branched alkyl.

Each Y is independently selected from N or C. If either Y is N, then R or U attached to Y is a lone
10 pair of electrons.

Z is CH, N, C(OCH₃), C(CH₃), C(NH₂), C(OH) or C(F).

Each U is independently selected from R or J.

Each J is independently selected from a (C₁-C₄)
15 straight chain or branched alkyl derivative substituted with T.

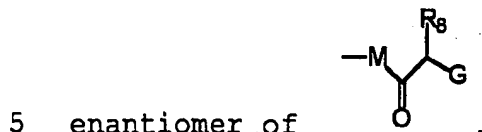
Each T is independently selected from either O(V) or N(H)(V).

Each V is independently selected from
20 C(O)N=C(R)(N(R)₂), wherein the two geminal R on the nitrogen are optionally bound to one another to form a 4-8 membered carbocyclic or heterocyclic ring.

When the two R components form a ring, it will be obvious to those skilled in the art that a terminal
25 hydrogen from each unfused R component will be lost. For example, if a ring structure is formed by binding those two R components together, one being -CH₃ and the other being -CH₂-CH₃, one terminal hydrogen on each R component (indicated in bold) will be lost. Therefore, the
30 resulting portion of the ring structure will have the formula -CH₂-CH₂-CH₂-.

Each K is independently selected from a (C₁-C₄) straight chain or branched alkyl derivative substituted with D, or -OP(O)(OH)₂.

Each D is independently selected from either



Each M is independently selected from either O or NH.

Each G is independently selected from NH₂, OH, or H.

10 Each R₈ is independently selected from H, OH, C(O)OH, (C₁-C₇)-straight or branched alkyl optionally substituted with N(R')₂, OR', CO₂R', CON(R')₂, or SO₂N(R⁵)₂; or a 5-6 membered carbocyclic, heterocyclic or heteroaryl ring system optionally substituted with

15 N(R')₂, OR', CO₂R', CON(R')₂, or SO₂N(R⁵)₂. When G forms a ring with R₈, it will be obvious to those skilled in the art that a terminal hydrogen from the unfused G and R₈ component will be lost. For example, if a ring structure is formed by binding the G and R₈ components together,

20 one being -NH₂ and the other being -CH₂-CH₂-CH₂-CH₃, one terminal hydrogen on each R component (indicated in bold) will be lost. Therefore, the resulting portion of the ring structure will have the formula -NH-CH₂-CH₂-CH₂-CH₂-.

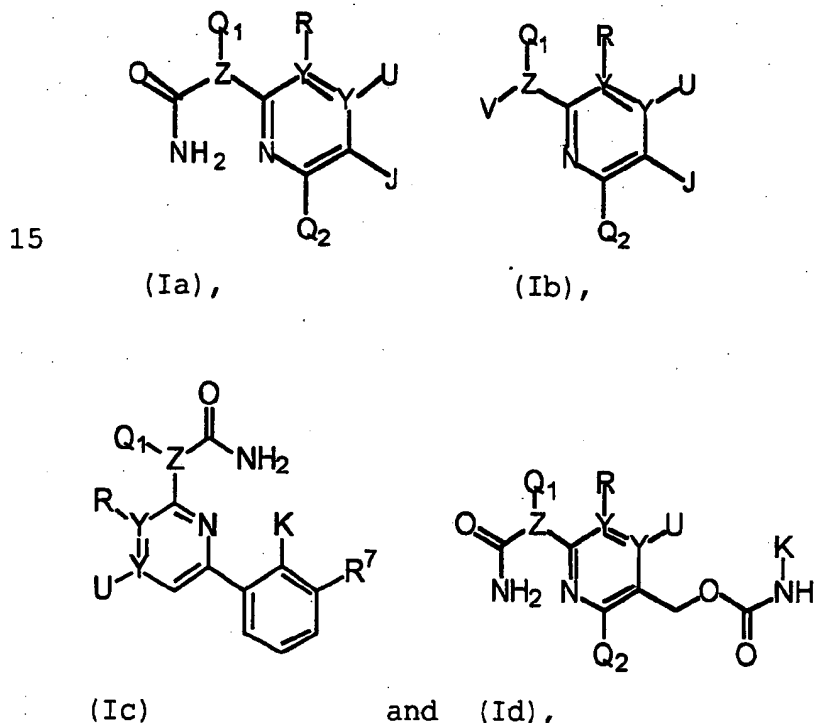
In another embodiment, the invention provides

25 pharmaceutical compositions comprising the p38 and/or ZAP70 inhibitors of this invention. These compositions may be utilized in methods for treating or preventing a variety of p38-mediated disorders, such as cancer, inflammatory diseases, autoimmune diseases, destructive

bone disorders, proliferative disorders, infectious diseases, viral diseases and neurodegenerative diseases or ZAP70-mediated disorders, including transplantation, autoimmune disease, cancer, multiple sclerosis, graft
 5 versus host disease, and Kawasaki syndrome. These compositions are also useful in methods for preventing cell death and hyperplasia and therefore may be used to treat or prevent reperfusion/ischemia in stroke, heart attacks, and organ hypoxia. The compositions are also
 10 useful in methods for preventing thrombin-induced platelet aggregation. Each of these above-described methods is also part of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

These compounds have the general formula:



20 wherein each of Q_1 and Q_2 are independently selected from a phenyl or 5-6 membered aromatic heterocyclic ring

system, or a 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring.

- 5 The rings that make up Q_1 are substituted with 1 to 4 substituents, each of which is independently selected from halo; C_1 - C_3 alkyl optionally substituted with NR'_2 , OR' , CO_2R' or $CONR'_2$; O -(C_1 - C_3)-alkyl optionally substituted with NR'_2 , OR' , CO_2R' or $CONR'_2$;
 10 NR'_2 ; OCF_3 ; CF_3 ; NO_2 ; CO_2R' ; $CONR'$; SR' ; $S(O_2)N(R')_2$; SCF_3 ; CN ; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$; $N(R')S(O_2)R^4$; $N(R')R^4$; $N(R^4)_2$; OR^4 ; $OC(O)R^4$; $OP(O)_3H_2$; or $N=C-N(R')_2$.

- The rings that make up Q_2 are optionally
 15 substituted with up to 4 substituents, each of which is independently selected from halogen; C_1 - C_3 straight or branched alkyl optionally substituted with R' , NR'_2 , OR' , CO_2R' , $S(O_2)N(R')_2$, $N=C-N(R')_2$, R^3 , $O-P(O_3)H_2$, or $CONR'_2$; O -(C_1 - C_3)-alkyl; O -(C_1 - C_3)-alkyl optionally substituted with
 20 NR'_2 , OR' , CO_2R' , $S(O_2)N(R')_2$, $N=CR'-N(R')_2$, R^3 , $OP(O_3)H_2$, or $CONR'_2$; NR'_2 ; OCF_3 ; CF_3 ; NO_2 ; CO_2R' ; $CONR'_2$; R^3 ; OR^3 ; NR^3_2 ; SR^3 ; $C(O)R^3$; $C(O)N(R')R^3$; $C(O)OR^3$; SR' ; $S(O_2)N(R')_2$; SCF_3 ; $N=CR'-N(R')_2$; OR^4 ; $O-CO_2R^4$; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$; $N(R')S(O_2)R^4$; $N(R')R^4$;
 25 $N(R^4)_2$; OR^4 ; $OC(O)R^4$; $OP(O)_3H_2$; K ; or CN .

- Each R' is independently selected from hydrogen; (C_1 - C_3)-alkyl; (C_2 - C_3)-alkenyl or alkynyl; phenyl or phenyl substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro,
 30 amino, hydroxy, methyl or ethyl; or a 5-6 membered

heterocyclic ring system optionally substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl.

Each R is independently selected from hydrogen,
 5 $-R^2$, $-N(R^2)_2$, $-OR^2$, SR^2 , $-C(O)-N(R^2)_2$, $-S(O_2)-N(R^2)_2$,
 $-C(O)-OR^2$ or $-C(O)R^2$ wherein two adjacent R are optionally bound to one another and, together with each Y to which they are respectively bound, form a 4-8 membered carbocyclic or heterocyclic ring.

10 Each R^2 is independently selected from hydrogen; or (C_1-C_3) -alkyl or (C_1-C_3) -alkenyl, each optionally substituted with $-N(R')_2$, $-OR'$, SR' , $-O-C(O)-N(R')_2$, $-C(O)-N(R')_2$, $-S(O_2)-N(R')_2$, $-C(O)-OR'$, $-NSO_2R^4$, $-NSO_2R^3$, $-C(O)N(R')(R^3)$, $-NC(O)R^4$, $-N(R')(R^3)$, $-N(R')(R^4)$, $-C(O)R^3$, $-C(O)N(R')(R^4)$, $-N(R^4)_2$, $-C(O)N=C(NH)_2$ or R^3 .
 15

Each R^3 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or heterocyclic ring systems each optionally substituted with R' , R^4 , $-C(O)R'$, $-C(O)R^4$, $-C(O)OR^4$ or $-K$; or an 8-10
 20 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R' , R^4 , $-C(O)R'$, $-C(O)R^4$, $-C(O)OR^4$ or $-K$.

25 Each R^4 is independently selected from R' ; (C_1-C_7) -straight or branched alkyl optionally substituted with R' , $N(R')_2$, OR' , CO_2R' , $CON(R')_2$, $SO_2N(R')_2$ or $SO_2N(R^5)_2$; or a 5-6 membered carbocyclic or heterocyclic ring system optionally substituted with $N(R')_2$, OR' ,
 30 CO_2R' , $CON(R')_2$, $SO_2N(R')_2$ or $SO_2N(R^5)_2$.

Each R^5 is independently selected from hydrogen, (C_1-C_3) -alkyl, or (C_1-C_3) -alkenyl; each optionally substituted with $-N(R')_2$, $-OR'$, SR' , $-C(O)-N(R')_2$, $-S(O_2)-N(R')_2$, $-C(O)-OR'$, $-N-S(O_2)(R')$, $-NSO_2R^6$, $-C(O)N(R')(R^6)$, $-NC(O)R'$, $-N(R')(R^6)$, $-C(O)R^6$, $-C(O)N=C(NH)_2$ or R^6 .

Each R^6 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or heterocyclic ring systems each optionally substituted with R' , $-C(O)R'$ or $-C(O)OR'$; or an 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R' , $-C(O)R'$ or $C(O)OR'$.

R^7 is selected from H, halogen, or a (C_1-C_3) straight chain or branched alkyl.

Each Y is independently selected from N or C. If either Y is N, then R or U attached to Y is a lone pair of electrons.

Z is CH, N, $C(OCH_3)$, $C(CH_3)$, $C(NH_2)$, $C(OH)$ or $C(F)$.

Each U is independently selected from R or J.

Each J is independently selected from a (C_1-C_4) straight chain or branched alkyl derivative substituted with T.

Each T is independently selected from either $O(V)$ or $N(H)(V)$.

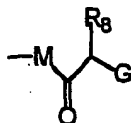
Each V is independently selected from $C(O)N=C(R)(N(R)_2)$, wherein the two geminal R on the

nitrogen are optionally bound to one another to form a 4-8 membered carbocyclic or heterocyclic ring.

When the two R components form a ring, it will be obvious to those skilled in the art that a terminal hydrogen from each unfused R component will be lost. For example, if a ring structure is formed by binding those two R components together, one being $-\text{CH}_3$ and the other being $-\text{CH}_2-\text{CH}_3$, one terminal hydrogen on each R component (indicated in bold) will be lost. Therefore, the resulting portion of the ring structure will have the formula $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$.

Each K is independently selected from a (C_1-C_4) straight chain or branched alkyl derivative substituted with D, or $-\text{OP}(\text{O})(\text{OH})_2$.

Each D is independently selected from either



enantiomer of

Each M is independently selected from either O or NH.

Each G is independently selected from NH_2 , OH, or H.

Each R_8 is independently selected from H, OH, $\text{C}(\text{O})\text{OH}$, (C_1-C_7) -straight or branched alkyl optionally substituted with $\text{N}(\text{R}')_2$, OR' , $\text{CO}_2\text{R}'$, $\text{CON}(\text{R}')_2$, or $\text{SO}_2\text{N}(\text{R}^5)_2$; or a 5-6 membered carbocyclic, heterocyclic or heteroaryl ring system optionally substituted with $\text{N}(\text{R}')_2$, OR' , $\text{CO}_2\text{R}'$, $\text{CON}(\text{R}')_2$, or $\text{SO}_2\text{N}(\text{R}^5)_2$. When G forms a ring with R_8 , it will be obvious to those skilled in the art that a terminal hydrogen from the unfused G and R_8 component will be lost. For example, if a ring structure

is formed by binding the G and R₈ components together, one being -NH₂ and the other being -CH₂-CH₂-CH₂-CH₃, one terminal hydrogen on each R component (indicated in bold) will be lost. Therefore, the resulting portion of the
5 ring structure will have the formula -NH-CH₂-CH₂-CH₂-CH₂-.

A heterocyclic ring system or a heterocyclic ring contains 1 to 4 heteroatoms, which are independently selected from N, O, and S. A substitutable nitrogen on an aromatic or non-aromatic heterocyclic ring may be
10 optionally substituted. N or S may also exist in oxidized form such as NO, SO and SO₂.

One having ordinary skill in the art will recognize that the maximum number of heteroatoms in a stable, chemically feasible heterocyclic ring, whether it
15 is aromatic or non-aromatic, is determined by the size of the ring, degree of unsaturation, and valence of the heteroatoms. In general, a heterocyclic ring may have one to four heteroatoms so long as the heterocyclic ring is chemically feasible and stable.

20 The term "chemically stable arrangement" or "chemically feasible and stable" as used herein, refers to a compound structure that renders the compound sufficiently stable to allow manufacture and administration to a mammal by methods known in the art.
25 Typically, such compounds are stable at a temperature of 40°C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

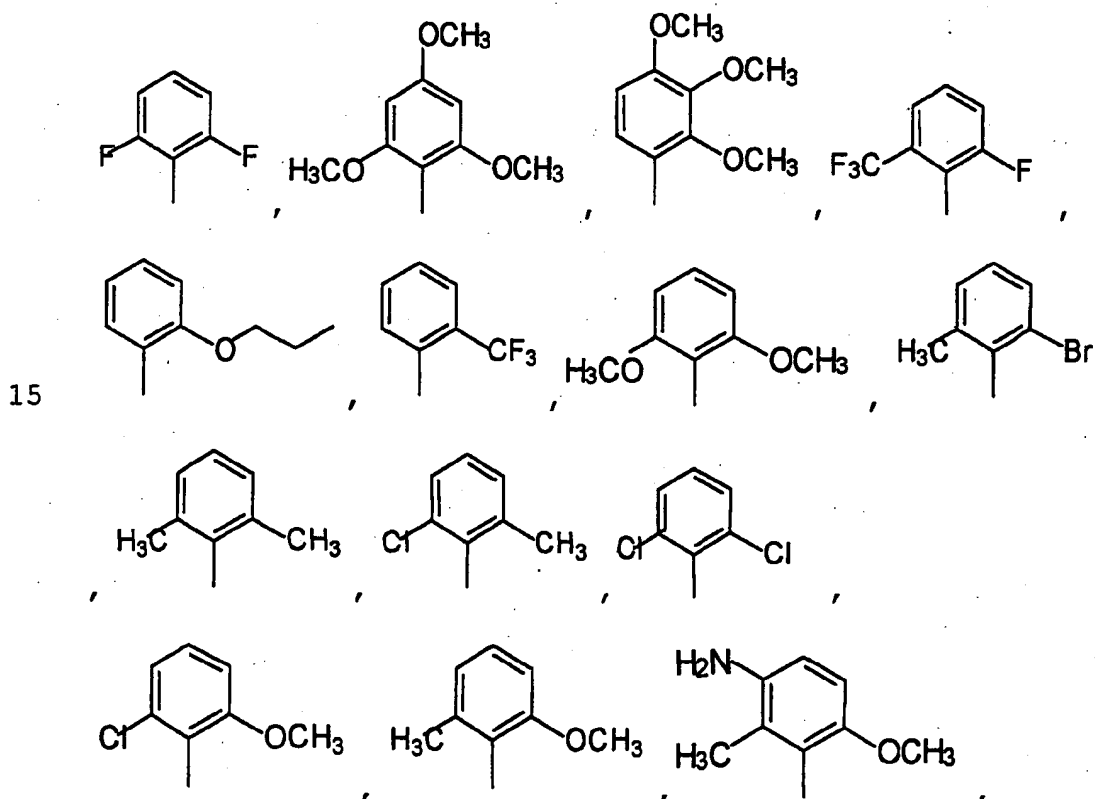
According to a preferred embodiment, Q₁ is selected from phenyl or pyridyl containing 1 to 3
30 substituents, wherein at least one of said substituents

is in the ortho position and said substituents are independently selected from chloro, fluoro, bromo, $-\text{CH}_3$, $-\text{OCH}_3$, $-\text{OH}$, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{O}(\text{CH}_2)_2\text{CH}_3$, NH_2 , 3,4-methylenedioxy, $-\text{N}(\text{CH}_3)_2$, $-\text{NH}-\text{S}(\text{O})_2\text{-phenyl}$, $-\text{NH}-\text{C}(\text{O})\text{O}-\text{CH}_2$ -

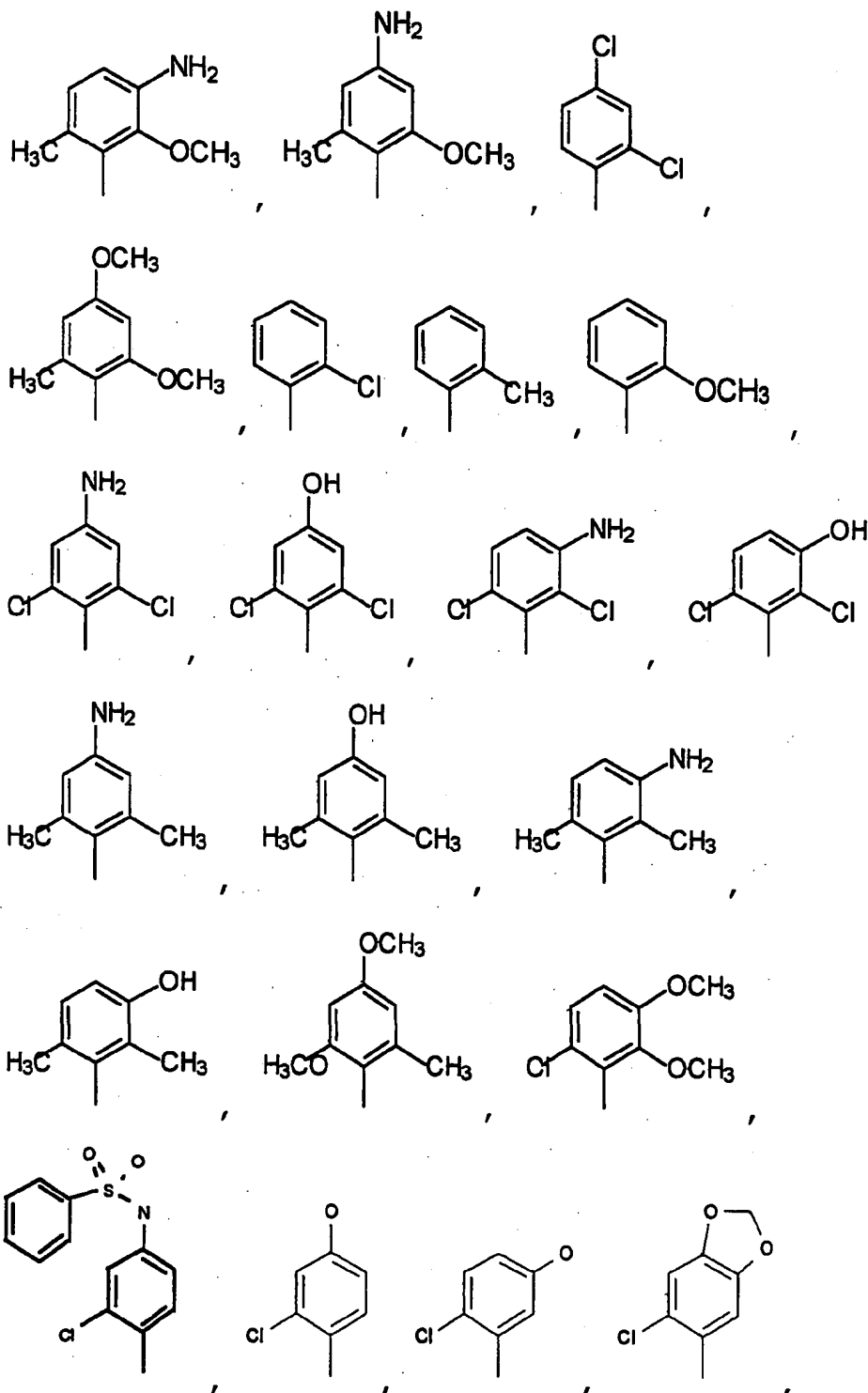
5 4-pyridine, $-\text{NH}-\text{C}(\text{O})\text{CH}_2\text{-morpholine}$, $-\text{NH}-\text{C}(\text{O})\text{CH}_2\text{-N}(\text{CH}_3)_2$, $-\text{NH}-\text{C}(\text{O})\text{CH}_2\text{-piperazine}$, $-\text{NH}-\text{C}(\text{O})\text{CH}_2\text{-pyrrolidine}$, $-\text{NH}-\text{C}(\text{O})\text{C}(\text{O})\text{-morpholine}$, $-\text{NH}-\text{C}(\text{O})\text{C}(\text{O})\text{-piperazine}$, $-\text{NH}-\text{C}(\text{O})\text{C}(\text{O})\text{-pyrrolidine}$, $-\text{O}-\text{C}(\text{O})\text{CH}_2\text{-N}(\text{CH}_3)_2$, or $-\text{O}-(\text{CH}_2)_2\text{-N}(\text{CH}_3)_2$.

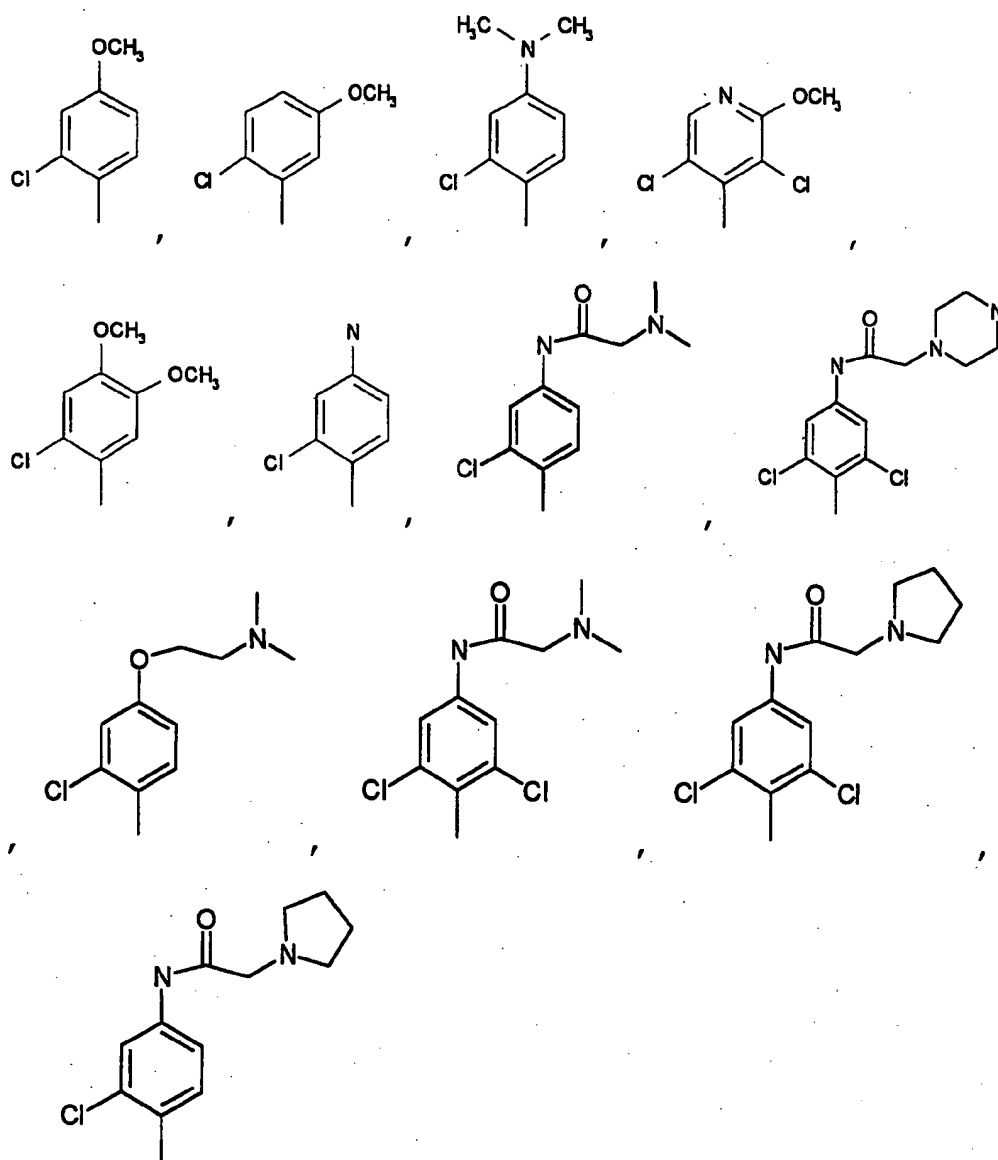
10 Even more preferred are phenyl or pyridyl containing at least 2 of the above-indicated substituents both being in the ortho position.

Some specific examples of preferred Q_1 are:



-16-





or

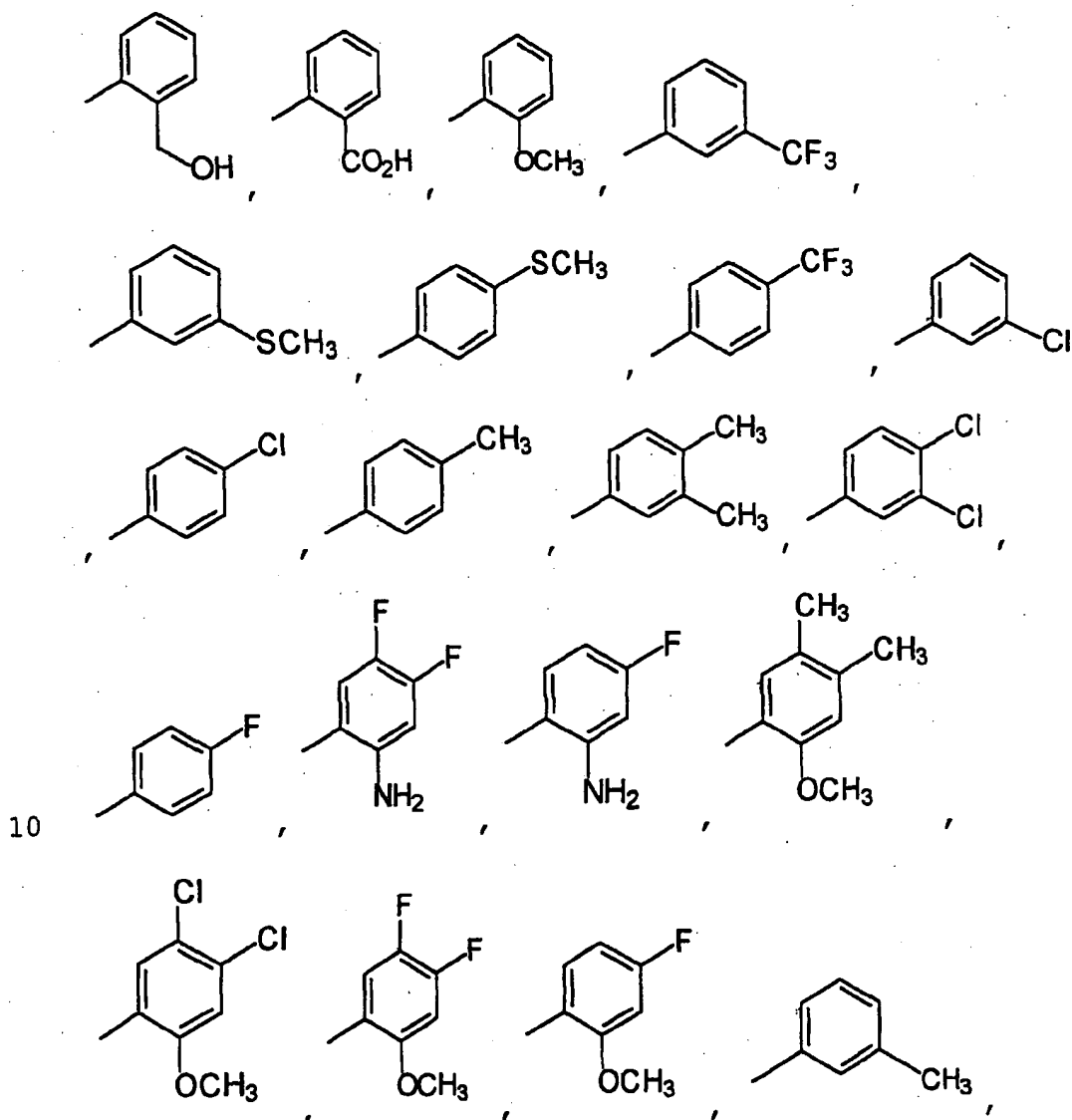
- 5 Most preferably, Q₁ is selected from 2-fluoro-6-trifluoromethylphenyl, 2,6-difluorophenyl, 2,6-dichlorophenyl, 2-chloro-4-hydroxyphenyl, 2-chloro-4-aminophenyl, 2,6-dichloro-4-aminophenyl, 2,6-dichloro-3-aminophenyl, 2,6-dimethyl-4-hydroxyphenyl, 2-methoxy-3,5-
 10 dichloro-4-pyridyl, 2-chloro-4,5 methylenedioxy phenyl, or 2-chloro-4-(N-2-morpholino-acetamido)phenyl.

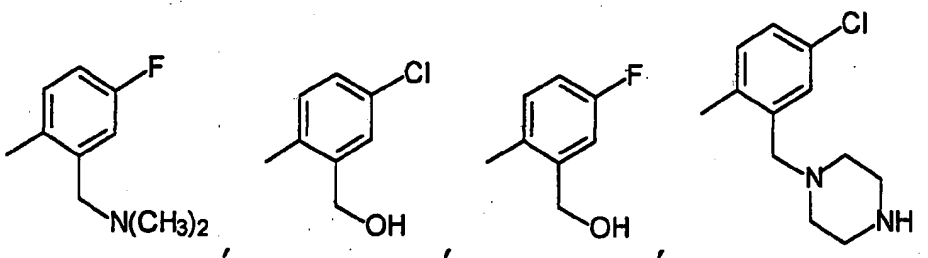
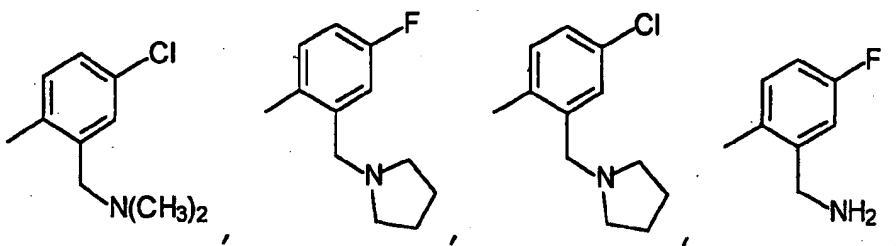
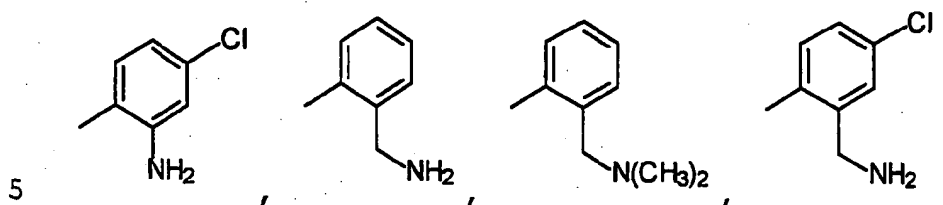
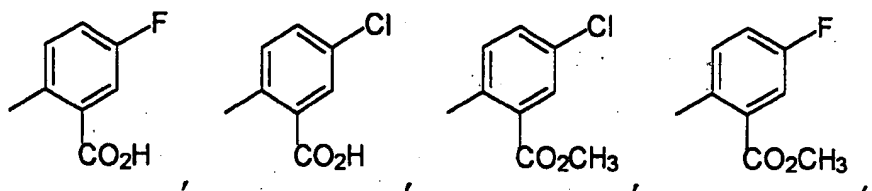
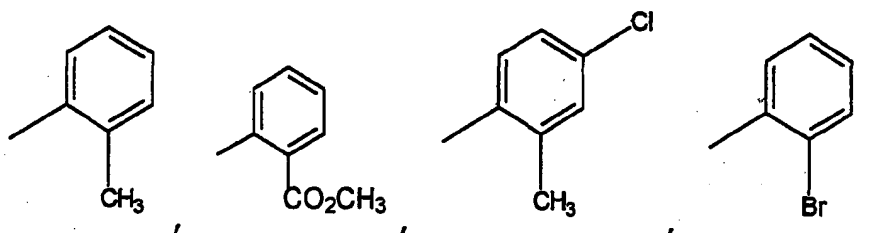
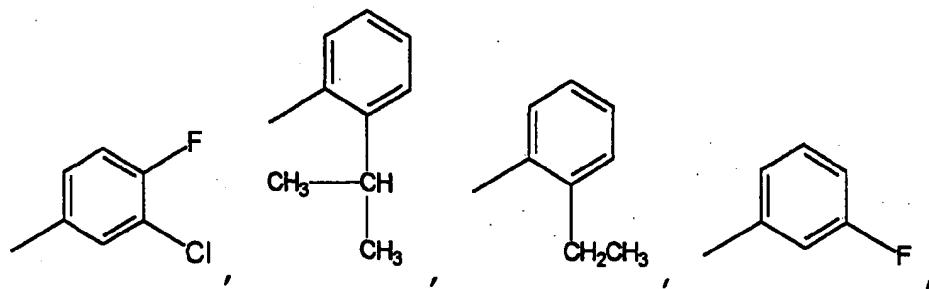
According to a preferred embodiment, Q₂ is phenyl, pyridyl or naphthyl containing 0 to 3

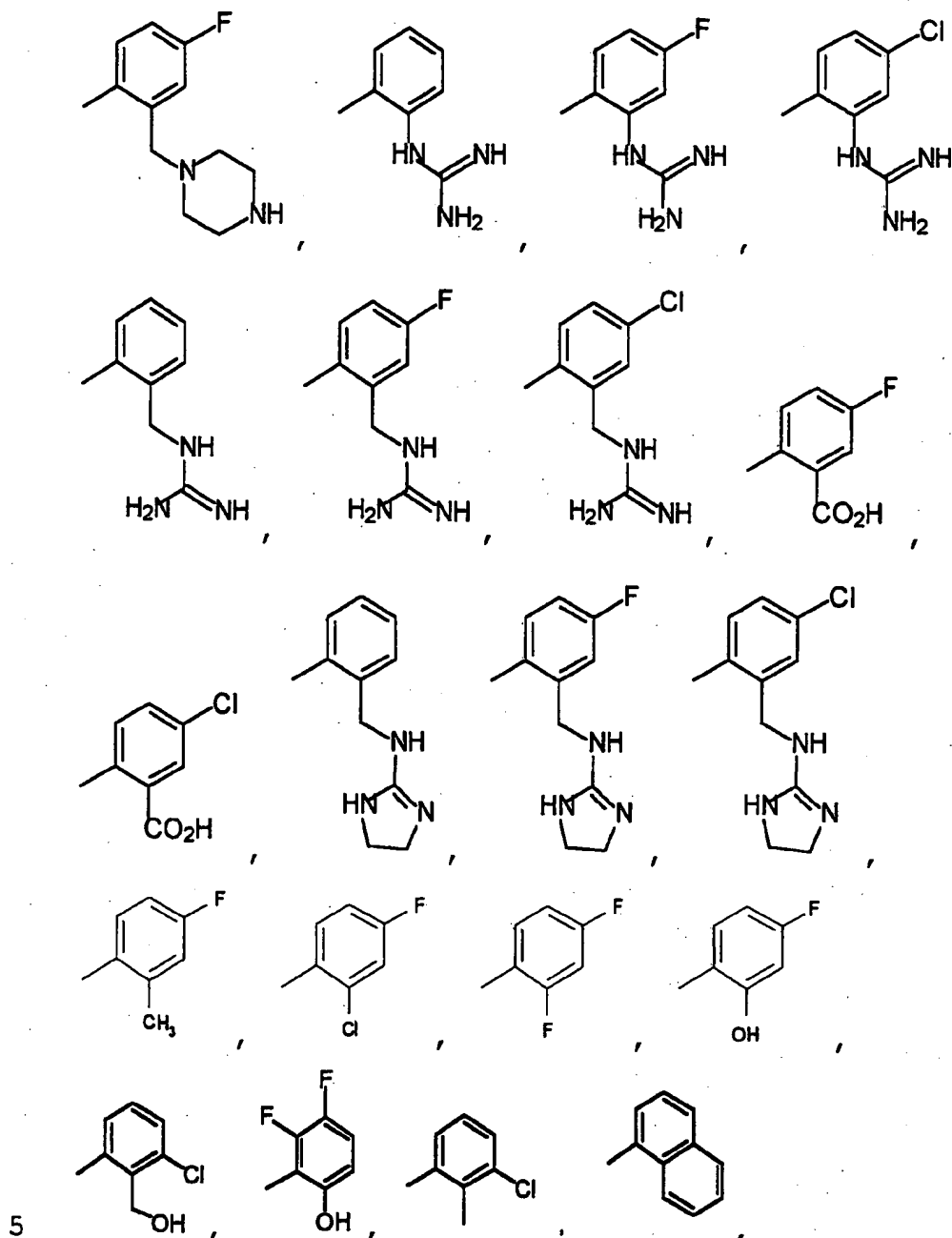
substituents, wherein each substituent is independently selected from chloro, fluoro, bromo, methyl, ethyl, isopropyl, $-\text{OCH}_3$, $-\text{OH}$, $-\text{NH}_2$, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{SCH}_3$, $-\text{OCH}_3$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OCH}_3$, $-\text{CH}_2\text{NH}_2$, $-\text{N}(\text{CH}_3)_2$, $-\text{CH}_2$ -pyrrolidine and

5 $-\text{CH}_2\text{OH}$.

Some specific examples of preferred Q_2 are:







unsubstituted 2-pyridyl or unsubstituted phenyl.

Most preferred are compounds wherein Q₂ is selected from phenyl, 2-isopropylphenyl, 3,4-dimethylphenyl, 2-ethylphenyl, 3-fluorophenyl, 2-methylphenyl, 3-chloro-4-fluorophenyl, 3-chlorophenyl, 2-carbomethoxyphenyl, 2-carboxyphenyl, 2-methyl-4-

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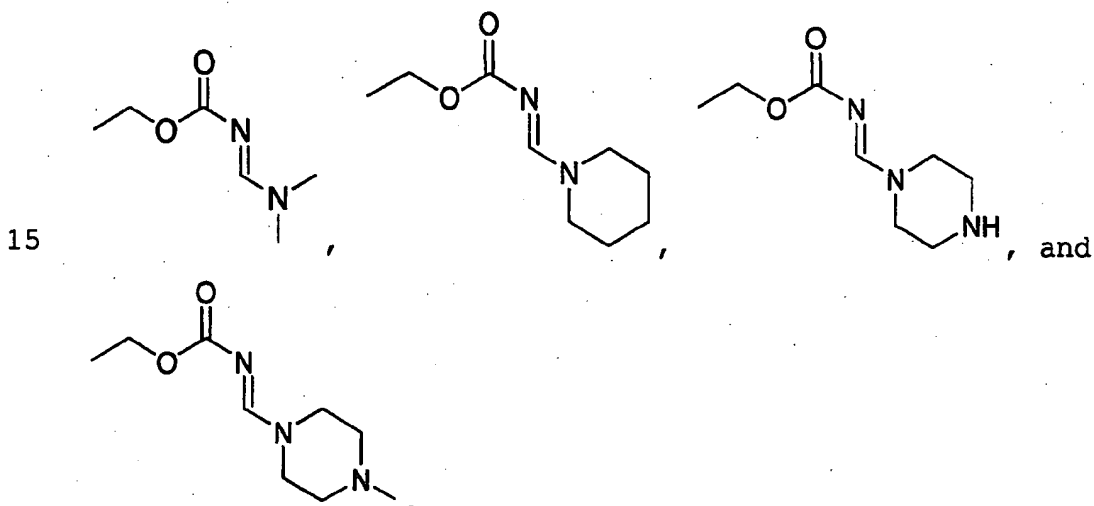
chlorophenyl, 2-bromophenyl, 2-pyridyl, 2-methylenehydroxyphenyl, 4-fluorophenyl, 2-methyl-4-fluorophenyl, 2-chloro-4-fluorophenyl, 2,4-difluorophenyl, 2-hydroxy-4-fluorophenyl, 2-methylenehydroxy-4-fluorophenyl, 1-naphthyl, 3-chloro-2-methylenehydroxy, 3-chloro-2-methyl, or 4-fluoro-2-methyl.

According to another preferred embodiment, R^7 is a halogen. In a more preferred embodiment, R^7 is Cl.

According to another preferred embodiment, each Y is C.

According an even more preferred embodiment, each Y is C and the R and U attached to each Y component is hydrogen.

Some specific examples of preferred J are:

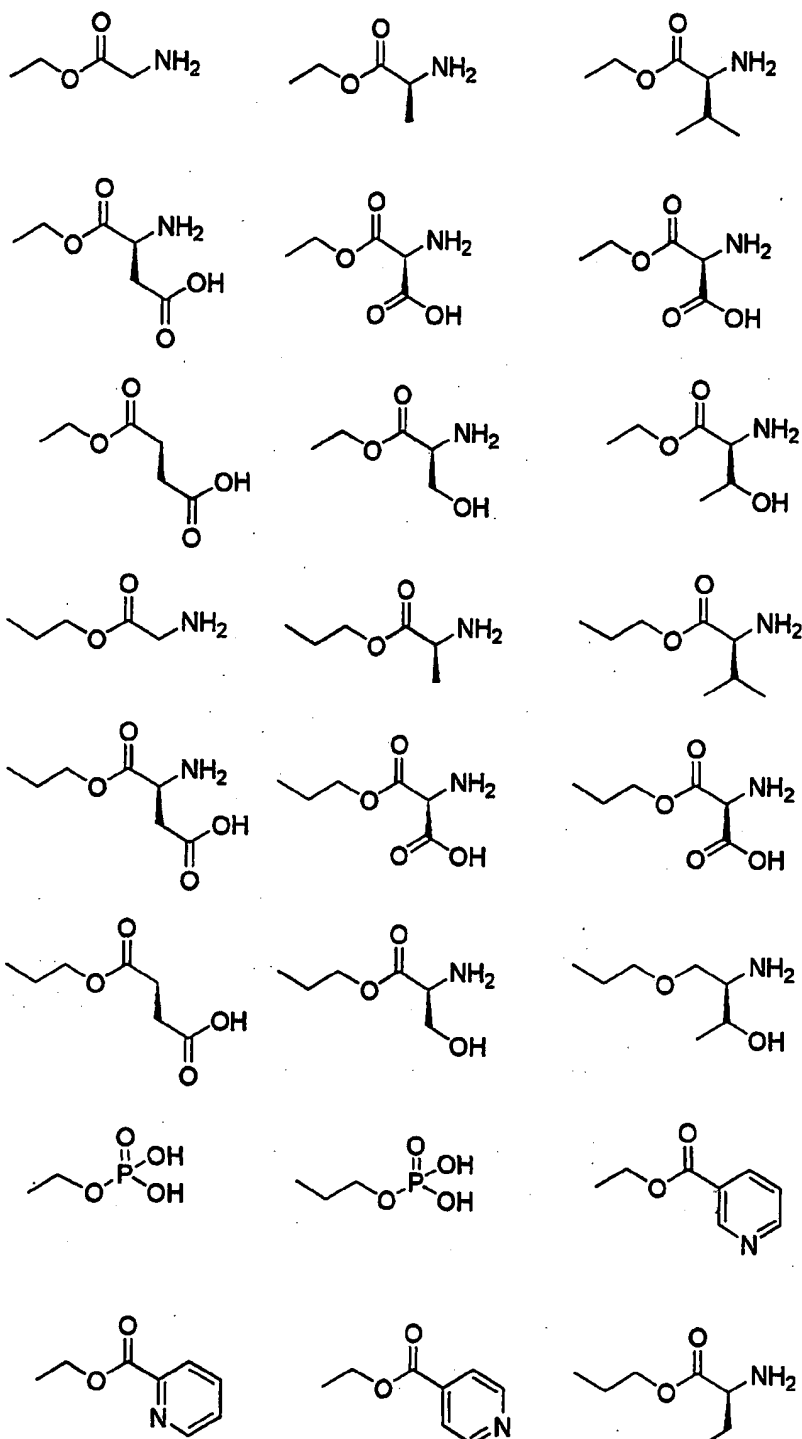


According to another preferred embodiment, K is a 0-4 atom chain terminating in an ester.

According to another preferred embodiment, M is O.

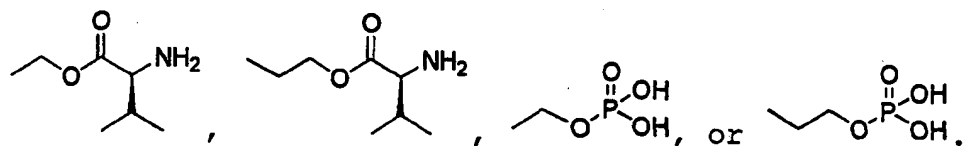
Some specific examples of preferred K are:

-22-



5

More preferably, K is selected from:



Some preferred embodiments are provided in
Tables 1 to 3 below:

5 Table 1.

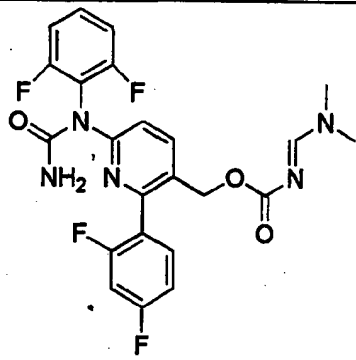
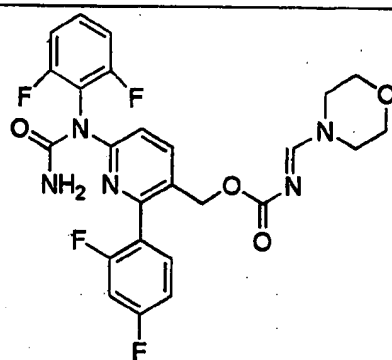
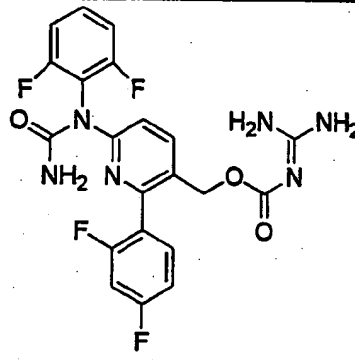
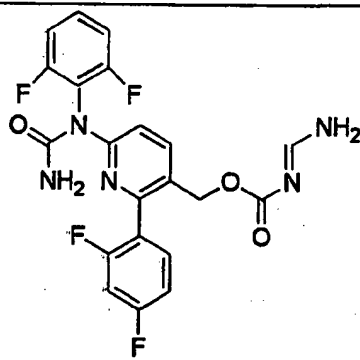
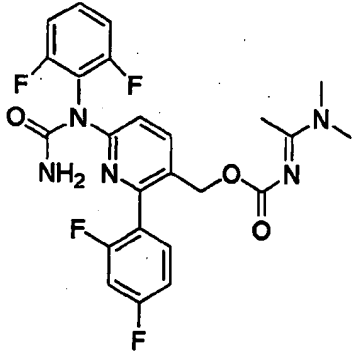
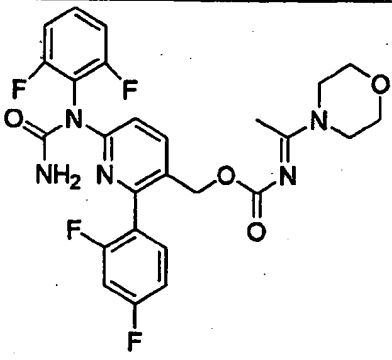
Cmpd Nmbr	Structure	Cmpd Nmbr	Structure
101		102	
103		104	
105		106	

Table 1 (cont.)

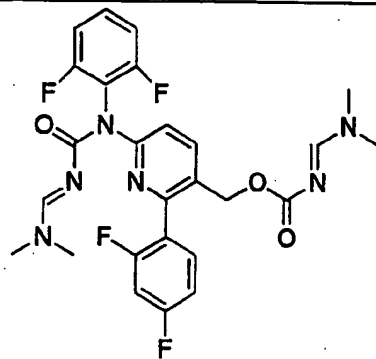
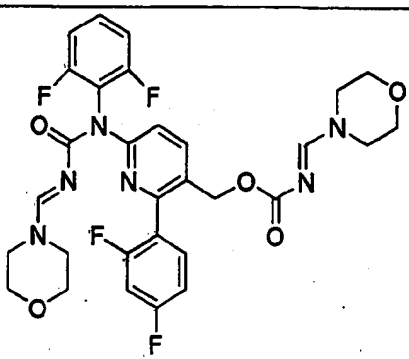
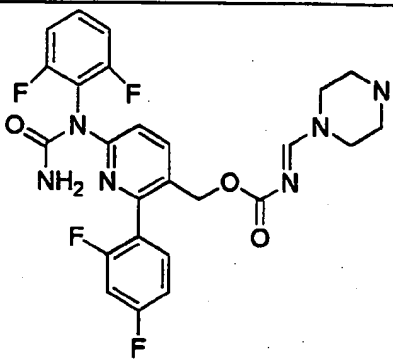
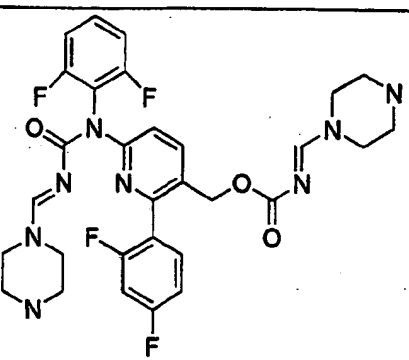
Cmpd Nmbr	Structure	Cmpd Nmbr	Structure
107		108	
109		110	

Table 2.

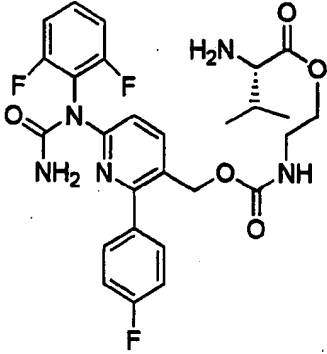
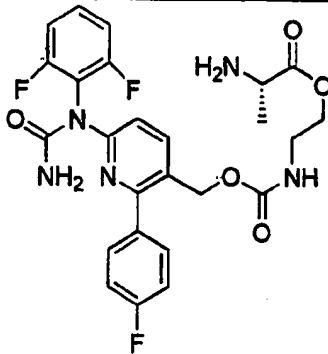
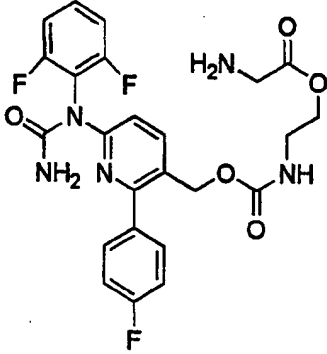
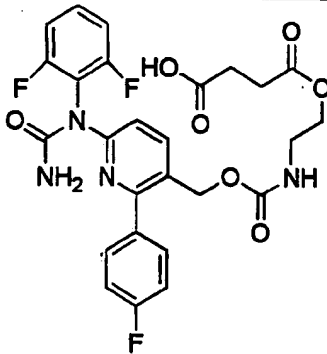
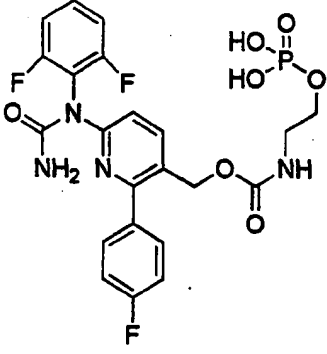
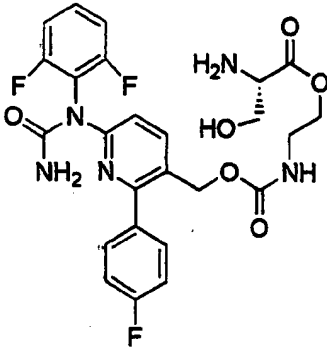
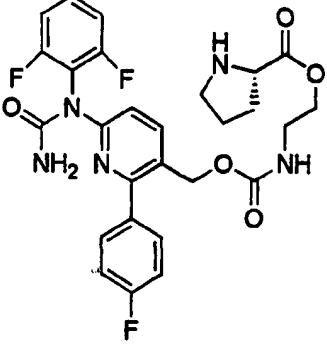
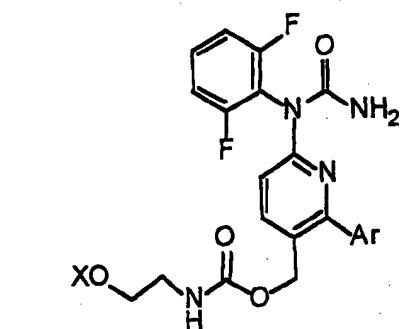
Cmpd Nbr	Structure	Cmpd Nbr	Structure
111		112	
113		114	
115		116	
117			

Table 3.

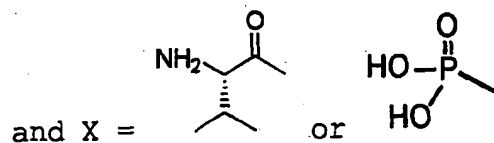
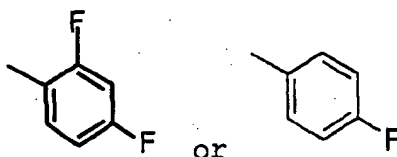
Cmpd Nbr	Structure	Cmpd Nbr	Structure
118		119	
120		121	
122		123	
124		125	

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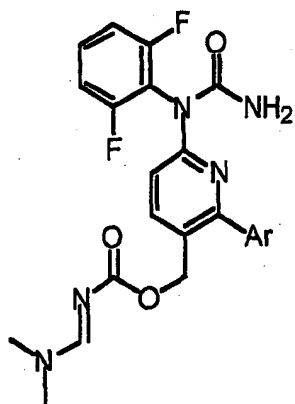
Particularly preferred embodiments include:



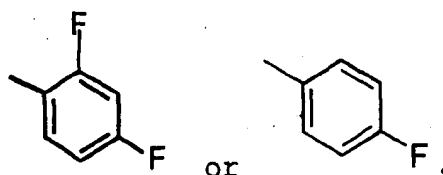
wherein Ar is



10. Particularly preferred embodiments also include:

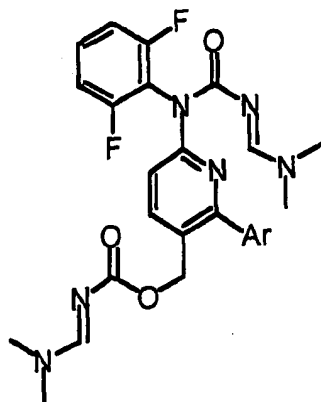


wherein Ar is

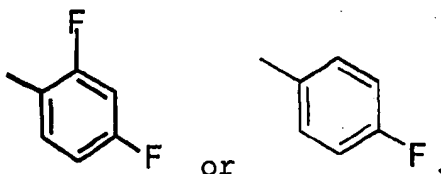


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Other particularly preferred embodiments
include:

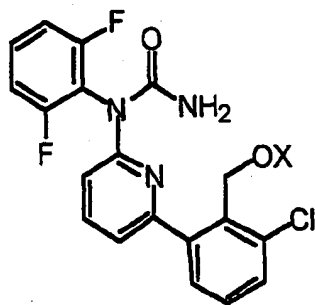


wherein Ar is



5

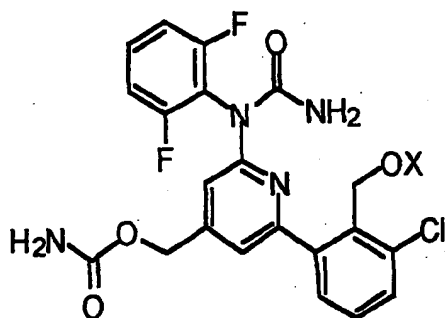
Other particularly preferred embodiments
include:



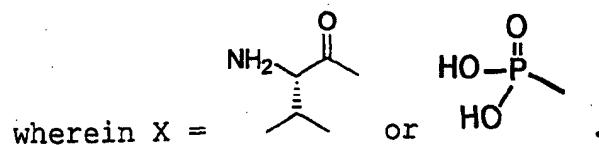
10 wherein X = or .

-29-

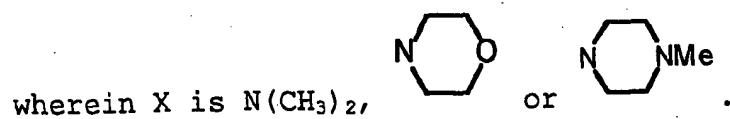
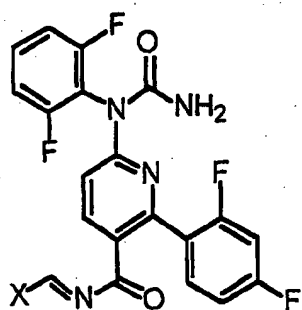
Other particularly preferred embodiments
include:



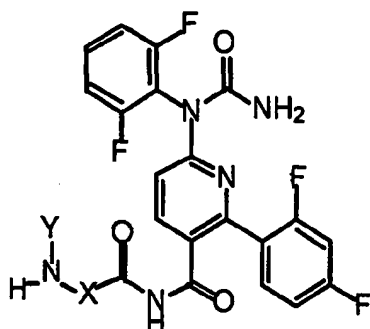
5



Other particularly preferred embodiments
10 include:

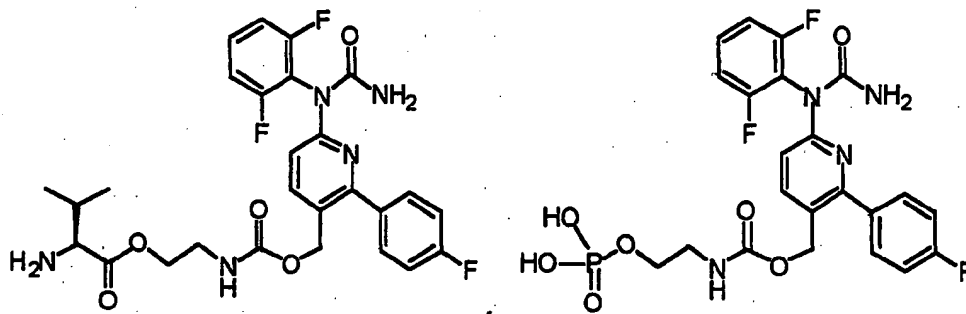


Other particularly preferred embodiments include:

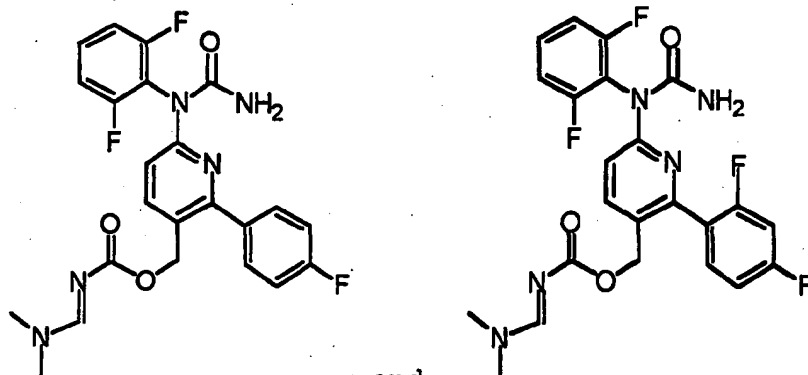


- 5 wherein Y = Me or H; and X = $(CH_2)_3$, $CH_2C(CH_3)_2CH_2$, $CH_2N(Me)C(O)CH_2$.

Some most preferred embodiments include:



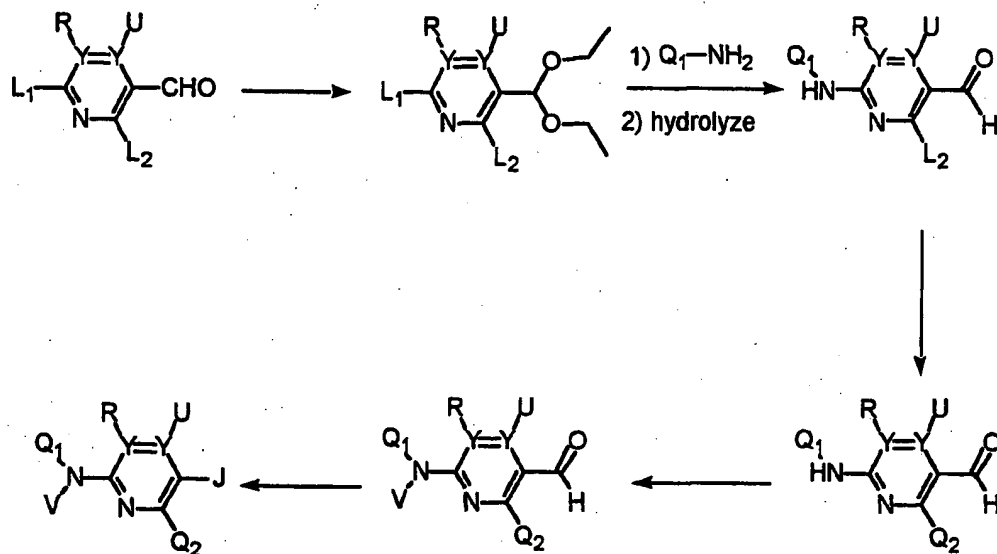
10



According to another embodiment, the present invention provides methods of producing the above-identified compounds of the formulae (Ia), (Ib), (Ic) or (Id). Representative synthesis schemes are depicted below. In all schemes, the L1 and L2 groups on the initial materials are meant to represent leaving groups ortho to the nitrogen atom in a heterocyclic ring. For example, compound A may be 2,6-dichloro-3 nitro pyridine.

Scheme 1

10



One having skill in the art will recognize Scheme 1 may be used to synthesize compounds having the general formula of (Ia), (Ib), (Ic) and (Id).

According to another embodiment of the invention, the activity of the p38 inhibitors of this invention may be assayed *in vitro*, *in vivo* or in a cell line. *In vitro* assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated p38. Alternate *in vitro* assays

quantitate the ability of the inhibitor to bind to p38 and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/p38 complex and determining the amount of radiolabel bound, or by running a competition experiment where new inhibitors are incubated with p38 bound to known radioligands.

Cell culture assays of the inhibitory effect of the compounds of this invention may determine the amounts of TNF, IL-1, IL-6 or IL-8 produced in whole blood or cell fractions thereof in cells treated with inhibitor as compared to cells treated with negative controls. Level of these cytokines may be determined through the use of commercially available ELISAs.

An *in vivo* assay useful for determining the inhibitory activity of the p38 inhibitors of this invention are the suppression of hind paw edema in rats with *Mycobacterium butyricum*-induced adjuvant arthritis. This is described in J.C. Boehm et al., J. Med. Chem., 39, pp. 3929-37 (1996), the disclosure of which is herein incorporated by reference. The p38 inhibitors of this invention may also be assayed in animal models of arthritis, bone resorption, endotoxin shock and immune function, as described in A. M. Badger et al., J. Pharmacol. Experimental Therapeutics, 279, pp. 1453-61 (1996), the disclosure of which is herein incorporated by reference.

The p38 inhibitors or pharmaceutical salts thereof may be formulated into pharmaceutical compositions for administration to animals or humans. These pharmaceutical compositions, which comprise an amount of p38 inhibitor effective to treat or prevent a

p38-mediated condition and a pharmaceutically acceptable carrier, are another embodiment of the present invention.

The term "p38-mediated condition", as used herein means any disease or other deleterious condition in which p38 is known to play a role. This includes conditions known to be caused by IL-1, TNF, IL-6 or IL-8 overproduction. Such conditions include, without limitation, inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, neurodegenerative diseases, allergies, reperfusion/ischemia in stroke, heart attacks, angiogenic disorders, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, thrombin-induced platelet aggregation, and conditions associated with prostaglandin endoperoxidase synthase-2.

Inflammatory diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, acute pancreatitis, chronic pancreatitis, asthma, allergies, and adult respiratory distress syndrome.

Autoimmune diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, atopic dermatitis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, psoriasis, or graft vs. host disease.

Destructive bone disorders which may be treated or prevented by the compounds of this invention include, but are not limited to, osteoporosis, osteoarthritis and multiple myeloma-related bone disorder.

5 Proliferative diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, and multiple myeloma.

10 Angiogenic disorders which may be treated or prevented by the compounds of this invention include solid tumors, ocular neovascularization, infantile haemangiomas.

Infectious diseases which may be treated or
15 prevented by the compounds of this invention include, but are not limited to, sepsis, septic shock, and Shigellosis.

Viral diseases which may be treated or prevented by the compounds of this invention include, but
20 are not limited to, acute hepatitis infection (including hepatitis A, hepatitis B and hepatitis C), HIV infection and CMV retinitis.

Neurodegenerative diseases which may be treated or prevented by the compounds of this invention include,
25 but are not limited to, Alzheimer's disease, Parkinson's disease, cerebral ischemias or neurodegenerative disease caused by traumatic injury.

"p38-mediated conditions" also include
ischemia/reperfusion in stroke, heart attacks, myocardial
30 ischemia, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, and thrombin-induced platelet aggregation.

In addition, p38 inhibitors of the instant invention are also capable of inhibiting the expression of inducible pro-inflammatory proteins such as prostaglandin endoperoxide synthase-2 (PGHS-2), also referred to as cyclooxygenase-2 (COX-2). Therefore, other "p38-mediated conditions" which may be treated by the compounds of this invention include edema, analgesia, fever and pain, such as neuromuscular pain, headache, cancer pain, dental pain and arthritis pain.

10 The diseases that may be treated or prevented by the p38 inhibitors of this invention may also be conveniently grouped by the cytokine (IL-1, TNF, IL-6, IL-8) that is believed to be responsible for the disease.

 Thus, an IL-1-mediated disease or condition includes rheumatoid arthritis, osteoarthritis, stroke, endotoxemia and/or toxic shock syndrome, inflammatory reaction induced by endotoxin, inflammatory bowel disease, tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, gout, traumatic arthritis, rubella arthritis, acute synovitis, diabetes, pancreatic β -cell disease and Alzheimer's disease.

 TNF-mediated disease or condition includes, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, cachexia secondary to infection, AIDS,

ARC or malignancy, keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis or pyresis. TNF-mediated diseases also include viral infections, such as HIV, CMV, influenza and herpes; and
5 veterinary viral infections, such as lentivirus infections, including, but not limited to equine infectious anemia virus, caprine arthritis virus, visna virus or maedi virus; or retrovirus infections, including feline immunodeficiency virus, bovine immunodeficiency
10 virus, or canine immunodeficiency virus.

IL-8 mediated disease or condition includes diseases characterized by massive neutrophil infiltration, such as psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury,
15 adult respiratory distress syndrome, thrombosis and glomerulonephritis.

In addition, the compounds of this invention may be used topically to treat or prevent conditions caused or exacerbated by IL-1 or TNF. Such conditions
20 include inflamed joints, eczema, psoriasis, inflammatory skin conditions such as sunburn, inflammatory eye conditions such as conjunctivitis, pyresis, pain and other conditions associated with inflammation.

According to another embodiment, the compounds
25 of this invention may be used to treat ZAP70-mediated conditions including, without limitation, organ or tissue rejection associated with transplantation, autoimmune disease, e.g., rheumatoid arthritis, systemic lupus erythematosus (SLE), psoriasis, Sjogren's Syndrome,
30 thyroiditis, pulmonary fibrosis, bronchiolitis obliterans, hemolytic anemia and Wegener's granulomatosis, cancer, including leukemia and lymphoma,

multiple sclerosis, graft versus host disease, and Kawasaki syndrome.

The ZAP70 inhibitors or pharmaceutical salts thereof may be formulated into pharmaceutical compositions for administration to animals or humans. These pharmaceutical compositions, which comprise an amount of ZAP70 inhibitor effective to treat or prevent a ZAP70-mediated condition and a pharmaceutically acceptable carrier, are another embodiment of the present invention.

In addition to the compounds of this invention, pharmaceutically acceptable salts of the compounds of this invention may also be employed in compositions to treat or prevent the above-identified disorders.

Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed

in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g., sodium and potassium), alkaline earth metal (e.g., magnesium), ammonium and N-(C1-4 alkyl)₄⁺ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional

and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other

dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added.

10 For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or

15 coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

20

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

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Topical application for the lower intestinal tract can be effected in a rectal suppository formulation

(see above) or in a suitable enema formulation.

Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives,

absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The amount of p38 or ZAP70 inhibitor that may
5 be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Preferably, the compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of
10 the inhibitor can be administered to a patient receiving these compositions.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the
15 activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of
20 inhibitor will also depend upon the particular compound in the composition.

According to another embodiment, the invention provides methods for treating or preventing a p38-mediated condition comprising the step of administering
25 to a patient one of the above-described pharmaceutical compositions. The term "patient", as used herein, means an animal, preferably a human.

Preferably, that method is used to treat or prevent a condition selected from inflammatory diseases,
30 autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, degenerative diseases, allergies, reperfusion/ischemia in

stroke, heart attacks, angiogenic disorders, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, and thrombin-induced platelet aggregation.

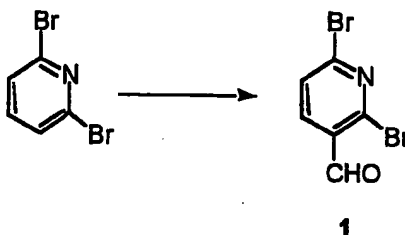
According to another embodiment, the inhibitors
5 of this invention are used to treat or prevent an IL-1, IL-6, IL-8 or TNF-mediated disease or condition. Such conditions are described above.

Depending upon the particular p38-mediated condition to be treated or prevented, additional drugs,
10 which are normally administered to treat or prevent that condition, may be administered together with the inhibitors of this invention. For example, chemotherapeutic agents or other anti-proliferative agents may be combined with the p38 inhibitors of this
15 invention to treat proliferative diseases.

Those additional agents may be administered separately, as part of a multiple dosage regimen, from the p38 inhibitor-containing composition. Alternatively, those agents may be part of a single dosage form, mixed
20 together with the p38 inhibitor in a single composition.

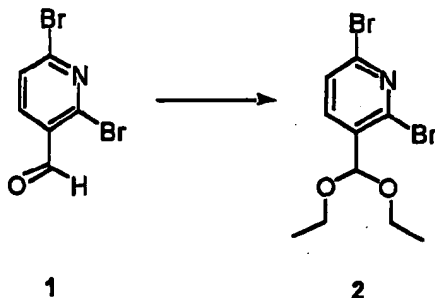
According to another embodiment, the invention provides methods for treating or preventing a ZAP70-mediated condition comprising the step of administering to a patient one of the above-described pharmaceutical
25 compositions.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be
30 construed as limiting this invention in any manner.

EXAMPLE 1Synthesis of p38 Inhibitor Compound 7

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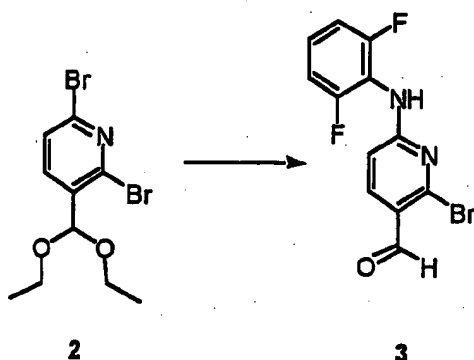
To a solution of LDA (60mmol, 40mLs) at -78°C , was added dropwise a solution of 2,6-dibromopyridine (40mmol, 9.48gms) in THF (30mLs, dried). The mixture was stirred at -78°C for 20 minutes. Ethyl formate (400mmol, 10 32.3mLs) was added and stirring was continued at -78°C for 2 hours. Saturated ammonium chloride (200mLs) was added and the mixture was warmed to room temperature. The reaction mixture was diluted with ethyl acetate and the organic layer was washed with aqueous acid and base. 15 The organic layer was dried and evaporated *in vacuo*. The resulting material was purified by flash chromatography on silica gel followed by eluting with 10% ethyl acetate in n-hexane to afford 1 (32mmol, 8.41gms) as a white solid.



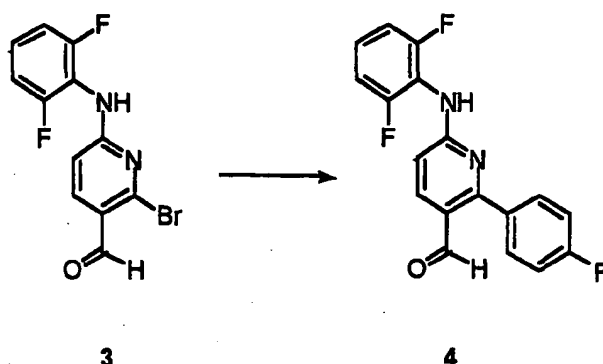
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A solution of 1 (776 mmol, 205.6 gms) and triethyl orthoformate (200 mL) dissolved in ethanol (750 mL) was refluxed overnight. The reaction mixture was

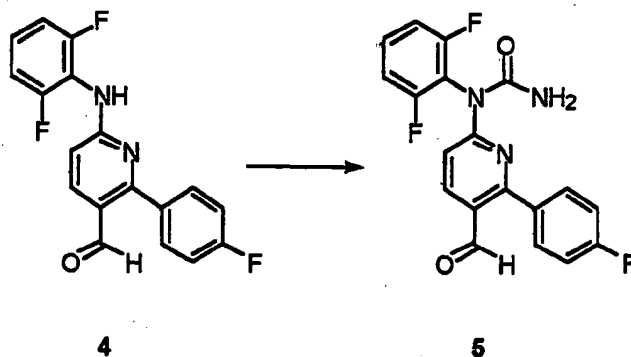
cooled, and evaporated in vacuo. The remaining red oil was dissolved in hexane and filtered over a plug of silica gel. The plug was eluted with 50% CH₂Cl₂/hexane. The filtrate was evaporated to afford 2 as an oil.



- 5
- 2
- 3
- To a suspension of 60% NaH (130 mmol, 5.20 g) and 2 (61.2 mmol, 20.76 g) in THF (100 mL) at reflux was added dropwise a solution of 2,6-difluoroaniline (61.3 mmol, 20 g) in THF (100 mL). After the aniline had been added, Pd(PPh₃)₄ (100 mg) was added. The mixture was refluxed for one hour and cooled. Hydrochloric acid (1N, 100 mL) was added and stirring was continued for one hour. The reaction mixture was extracted with CH₂Cl₂.
- 10
- 15 The organic extract was dried and evaporated in vacuo. The resulting material was dissolved in a minimal amount of CH₂Cl₂ and hexane was added. The solution was cooled precipitating 3 as a yellow solid.

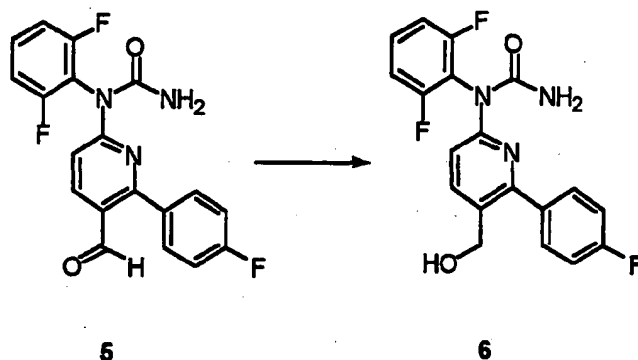


p-fluorophenylboronic acid (57.5 mmol, 8.05 g), and **3** (46.9 mmol, 14.70 g) were dissolved in a
5 dimethoxyethane (300 mL). Cesium fluoride (68.6 mmol, 10.42 g) and tetrakis(triphenylphosphine)palladium (0) (100 mgs) were added to the solution and the suspension was allowed to reflux overnight. The reaction mixture was poured into water and extracted with CH_2Cl_2 . The
10 organic extract was washed with 1N NaOH, dried with MgSO_4 , and filtered over a plug of silica gel. The plug was eluted with CH_2Cl_2 and the filtrate was evaporated in *vacuo*. The resulting yellow solid was triturated with 50% CH_2Cl_2 /hexane to afford **4** (9.50g, 62%) as a yellow
15 solid.



A solution of **4** (70.1 mmol, 23.01 g) in toluene (250 mL) was combined with a 20% solution of phosgene in
20 toluene (151 mmol, 80 mL) and heated to reflux for two

hours. The reaction was cooled and poured into ammonium hydroxide. The mixture was stirred for five minutes and extracted with methylene chloride. The organic extract was dried and filtered over a plug of silica gel. The plug was eluted with methylene chloride to remove residual starting material. It was then eluted with 50% ethyl acetate/methylene chloride to obtain 5. The filtrate was evaporated in vacuo to afford 5 (21.38 g, 86%) as a white solid.



10

Sodium borohydride (36.5 mmol, 1.38 g) was added to a solution of 5 (60.0 mmol, 21.38 g) in THF (100 mL) and the solution was stirred for one hour at 0°C and then two hours at room temperature. The reaction was poured into 1N HCl and extracted with methylene chloride. The organic extract was dried and filtered over a plug of silica gel. The plug was eluted with 5% ethyl acetate/methylene chloride to remove residual starting material. It was then eluted with ethyl acetate to obtain 6. The filtrate was evaporated to afford 6 as a white solid.

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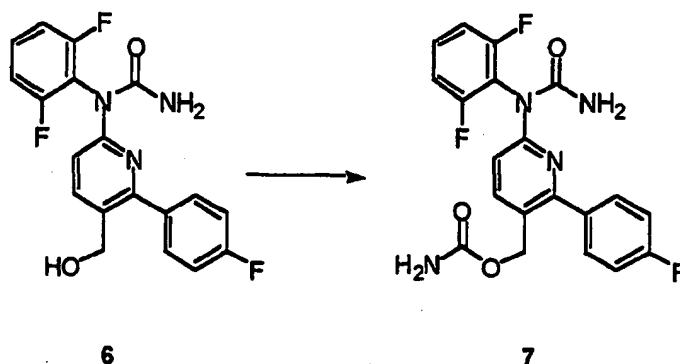
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The spectral data for compound 6 was:

^1H NMR (500 MHz, CDCl_3) δ 7.90 (d, 1H), 7.60 (d, 2H), 7.5-7.3 (m, 5H), 6.30 (d, 2H), 4.5 (s, 2H), 2.3 (s, 2H).

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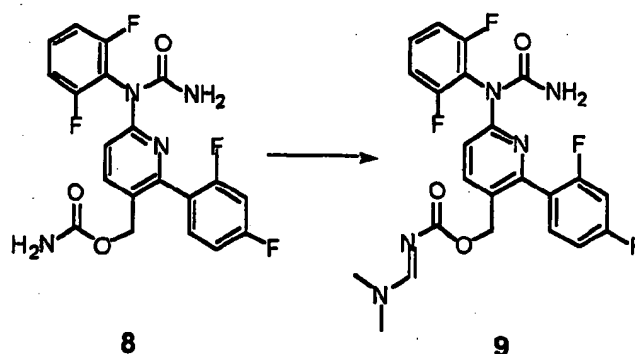
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A solution of 6 (2.79 mmol, 1.00 g) and p-nitrophenyl chloroformate (5.56 mmol, 1.12 g) was cooled to 0°C. Triethylamine (14.3 mmol, 2.0 mL) was added and the solution was stirred for 15 minutes and poured into ammonium hydroxide. The solution mixture was poured into water and extracted with methylene chloride. The organic extract was washed with saturated aqueous sodium bicarbonate, dried, and evaporated in vacuo to afford 7 (730 mg, 65%) as a white solid.

EXAMPLE 2

Synthesis of p38 Inhibitor Prodrugs 9 and 10



A mixture of 8 (1.0 g, 2.30 mmol) and N,N-dimethylformamide dimethyl acetal (1.01 g, 6.91 mmol) in 10 mL of toluene was heated to 80°C for 20 minutes. The

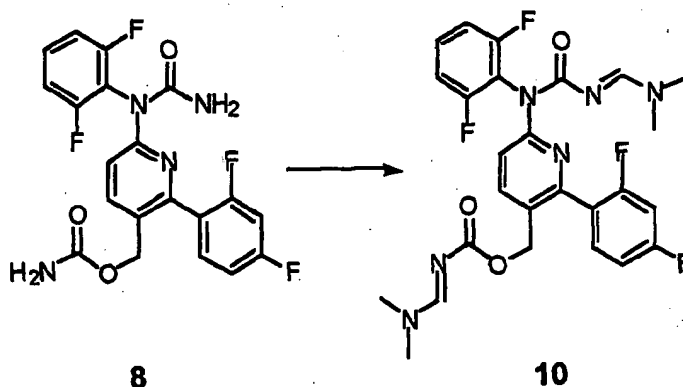
resulting solution was cooled to room temperature.

Normal workup followed by chromatography on silica gel

(hexane/EtOAc: 10/4) gave amidine 9 (compound 101 of

Table 1) as a white solid. The spectral data for

5 compound 9 was: ^1H NMR (500 MHz, CDCl_3) δ 8.3 (s, 1H), 7.7 (d, 1H), 7.5-7.4 (m, 1H), 7.1-7.0 (m, 1H), 6.95-6.85 (t, 2H), 6.85-6.75 (m, 1H), 6.45-6.4 (d, 1H), 6.2 (s, 1H), 4.95 (s, 2H), 3.05 (s, 3H), 2.95 (s, 3H).



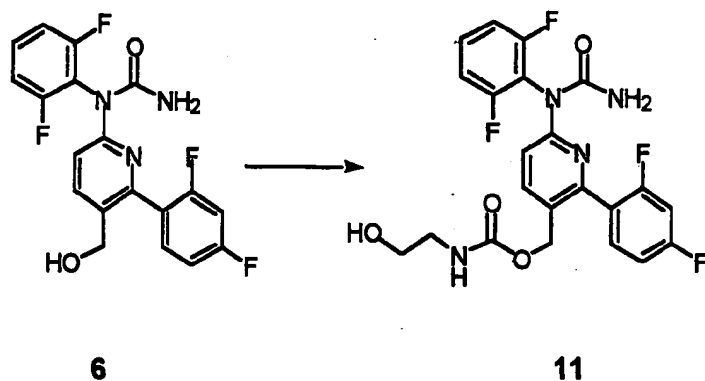
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A mixture of 8 (1.0 g, 2.30 mmol) and N,N-dimethylformamide dimethyl acetal (3.3 g, 22.4 mmol) in 10 mL of toluene was heated to 80°C for 90 minutes. The resulting solution was cooled to room temperature.

15 Normal workup followed by chromatography on silica gel (hexane/EtOAc: 2/1) gave bis-amidine 10 (compound 107 of Table 1) as a white solid. The spectral data for

20 compound 10 was: ^1H NMR (500 MHz, CDCl_3) δ 8.4 (s, 1H), 8.3 (s, 1H), 8.05-7.95 (s, 1H), 7.15-7.05 (m, 2H), 6.85-6.75 (t, 2H), 6.75-6.65 (m, 4H), 4.95 (s, 2H), 3.0-2.95 (d, 9H), 2.65 (s, 3H).

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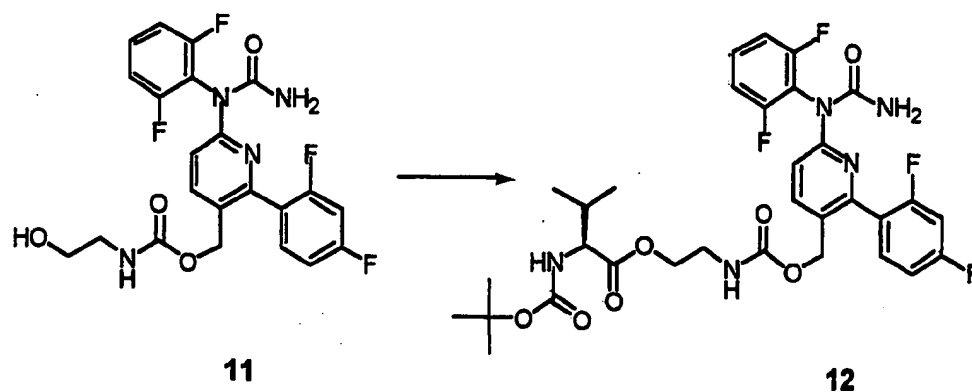
EXAMPLE 3Synthesis of p38 Inhibitor Prodrug 13

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To a mixture of 6 (1.25gm, 3.35mmol) and 4-nitrophenyl chloroformate (0.81gm, 4.02mmol) in tetrahydrofuran (30mL) was added triethylamine (1.16mL, 8.38mmol) dropwise at 0°C. The resulting slurry was allowed to stir at 0°C for 30 minutes. Ethanolamine (0.6mL, 10.0mmol) was added and the solution was stirred at 0°C for 30 minutes. Normal work-up followed by chromatography on silica (hexane/acetone: 10/4) gave 11 (1.03gm, 2.23mmol) as a white solid. ¹H NMR (500 MHz, CDCl₃) 7.75 (d, 1H), 7.65-7.55 (m, 2H), 7.5-7.4 (m, 1H), 7.25-7.15 (t, 2H), 7.15-7.05 (t, 2H), 6.4 (d, 1H), 5.2-5.1 (bs, 1H), 5.15 (s, 2H), 3.75-3.65 (t, 2H), 3.4-3.3 (m, 2H).

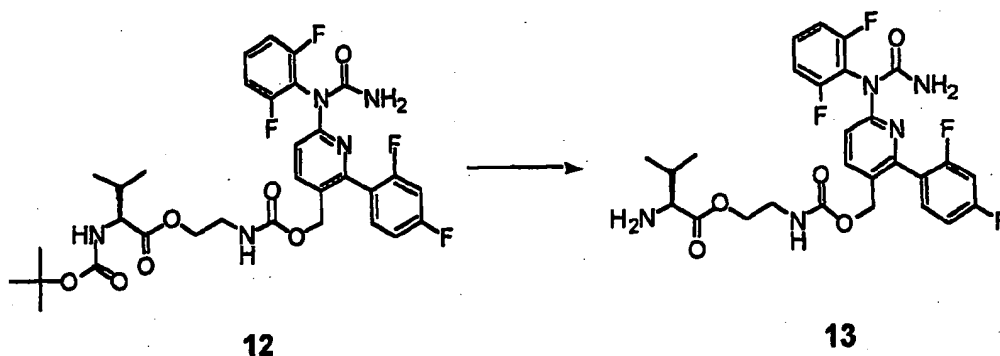
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A mixture of 11 (1.03gm, 2.23mmol), (L)-BOC-Val-OH (0.97gm, 4.46mmol), and 1-(3-dimethylaminopropyl) 3-ethylcarbodiimide hydrochloride in methylene chloride (30mL) was stirred at room temperature for 1.5 hours.

- 5 Normal work-up followed by chromatography on silica (hexane/acetone: 10/4) gave Val deriv. 12 (1.38gms, 2.09mmol) as a white solid. ¹H NMR (500 MHz, CDCl₃) 7.75 (d, 1H), 7.65-7.55 (m, 2H), 7.5-7.4 (m, 1H), 7.25-7.15 (t, 2H), 7.15-7.05 (t, 2H), 6.4 (d, 1H), 5.40-5.35 (bs, 1H), 5.05 (s, 2H), 5.00-4.95 (d, 1H), 4.4-4.3 (m, 1H), 4.25-4.15 (m, 1H), 4.15-4.05 (m, 1H), 3.55-3.45 (m, 2H), 2.15-2.05 (m, 1H), 1.45 (s, 9H), 1.0-0.85 (m, 6H).



15

- To a solution of 12 (1.38gms, 2.09mmol) in methylene chloride (20mLs) was added trifluoroacetic acid (10mLs). The solution was allowed to stir at room temperature for 1 hour. Normal work-up gave a white solid that was converted to its hydrochloride salt to give 13 (compound 111 of Table 2; 0.61gms, 1.02mmol) as a white solid. The spectral data for compound 13 was: ¹H NMR (500 MHz, CDCl₃) 7.65 (d, 1H), 7.55-7.45 (m, 2H), 7.4-7.3 (m, 1H), 7.15-7.05 (m, 2H), 7.05-6.95 (m, 2H), 6.35 (d, 1H), 5.05-5.00 (bs, 1H), 4.95 (s, 2H), 4.15-4.05 (m, 2H), 25

3.45-3.25 (m, 2H), 3.2 (s, 1H), 1.95-1.85 (m, 1H), 0.90-0.75 (m, 6H).

EXAMPLE 4

Cloning of p38 Kinase in Insect Cells

- 5 Two splice variants of human p38 kinase, CSBP1 and CSBP2, have been identified. Specific oligonucleotide primers were used to amplify the coding region of CSBP2 cDNA using a HeLa cell library (Stratagene) as a template. The polymerase chain
- 10 reaction product was cloned into the pET-15b vector (Novagen). The baculovirus transfer vector, pVL-(His)6-p38 was constructed by subcloning a XbaI-BamHI fragment of pET15b-(His)6-p38 into the complementary sites in plasmid pVL1392 (Pharmingen).
- 15 The plasmid pVL-(His)6-p38 directed the synthesis of a recombinant protein consisting of a 23-residue peptide (MGSSHHHHHSSGLVPRGSHMLE, where LVPRGS represents a thrombin cleavage site) fused in frame to the N-terminus of p38, as confirmed by DNA sequencing and
- 20 by N-terminal sequencing of the expressed protein. Monolayer culture of *Spodoptera frugiperda* (Sf9) insect cells (ATCC) was maintained in TNM-FH medium (Gibco BRL) supplemented with 10% fetal bovine serum in a T-flask at 27°C. Sf9 cells in log phase were co-transfected with
- 25 linear viral DNA of *Autographa californica* nuclear polyhedrosis virus (Pharmingen) and transfer vector pVL-(His)6-p38 using Lipofectin (Invitrogen). The individual recombinant baculovirus clones were purified by plaque assay using 1% low melting agarose.

EXAMPLE 5Expression and Purification of Recombinant p38 Kinase

Trichoplusia ni (Tn-368) High-Five™ cells (Invitrogen) were grown in suspension in Excel-405 protein free medium (JRH Bioscience) in a shaker flask at 27°C. Cells at a density of 1.5×10^6 cells/ml were
5 infected with the recombinant baculovirus described above at a multiplicity of infection of 5. The expression level of recombinant p38 was monitored by immunoblotting using a rabbit anti-p38 antibody (Santa Cruz Biotechnology). The cell mass was harvested 72 hours
10 after infection when the expression level of p38 reached its maximum.

Frozen cell paste from cells expressing the (His)₆-tagged p38 was thawed in 5 volumes of Buffer A (50 mM NaH₂PO₄, pH 8.0, 200 mM NaCl, 2mM β-Mercaptoethanol, 10%
15 Glycerol and 0.2 mM PMSF). After mechanical disruption of the cells in a microfluidizer, the lysate was centrifuged at 30,000 x g for 30 minutes. The supernatant was incubated batchwise for 3-5 hours at 4°C with Talon™ (Clontech) metal affinity resin at a ratio of
20 1 ml of resin per 2-4 mgs of expected p38. The resin was settled by centrifugation at 500 x g for 5 minutes and gently washed batchwise with Buffer A. The resin was slurried and poured into a column (approx. 2.6 x 5.0 cm) and washed with Buffer A + 5 mM imidazole.

25 The (His)₆-p38 was eluted with Buffer A + 100 mM imidazole and subsequently dialyzed overnight at 4°C against 2 liters of Buffer B, (50 mM HEPES, pH 7.5, 25 mM β-glycerophosphate, 5% glycerol, 2mM DTT). The His₆ tag was removed by addition of at 1.5 units thrombin

(Calbiochem) per mg of p38 and incubation at 20°C for 2-3 hours. The thrombin was quenched by addition of 0.2 mM PMSF and then the entire sample was loaded onto a 2 ml benzamidine agarose (American International Chemical) column.

The flow through fraction was directly loaded onto a 2.6 x 5.0 cm Q-Sepharose (Pharmacia) column previously equilibrated in Buffer B + 0.2 mM PMSF. The p38 was eluted with a 20 column volume linear gradient to 0.6M NaCl in Buffer B. The eluted protein peak was pooled and dialyzed overnight at 4°C vs. Buffer C (50 mM HEPES pH 7.5, 5% glycerol, 50 mM NaCl, 2 mM DTT, 0.2 mM PMSF).

The dialyzed protein was concentrated in a Centriprep (Amicon) to 3-4 ml and applied to a 2.6 x 100 cm Sephacryl S-100HR (Pharmacia) column. The protein was eluted at a flow rate of 35 ml/hr. The main peak was pooled, adjusted to 20 mM DTT, concentrated to 10-80 mgs/ml and frozen in aliquots at -70°C or used immediately.

EXAMPLE 6

Activation of p38

p38 was activated by combining 0.5 mg/ml p38 with 0.005 mg/ml DD-double mutant MKK6 in Buffer B + 10mM MgCl₂, 2mM ATP, 0.2mM Na₂VO₄ for 30 minutes at 20°C. The activation mixture was then loaded onto a 1.0 x 10 cm MonoQ column (Pharmacia) and eluted with a linear 20 column volume gradient to 1.0 M NaCl in Buffer B. The activated p38 eluted after the ADP and ATP. The activated p38 peak was pooled and dialyzed against buffer B + 0.2mM Na₂VO₄ to remove the NaCl. The dialyzed protein

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was adjusted to 1.1M potassium phosphate by addition of a 4.0M stock solution and loaded onto a 1.0 x 10 cm HIC (Rainin Hydropore) column previously equilibrated in Buffer D (10% glycerol, 20mM β -glycerophosphate, 2.0mM DTT) + 1.1MK₂HPO₄. The protein was eluted with a 20 column volume linear gradient to Buffer D + 50mM K₂HPO₄. The double phosphorylated p38 eluted as the main peak and was pooled for dialysis against Buffer B + 0.2mM Na₂VO₄. The activated p38 was stored at -70°C.

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EXAMPLE 7

p38 Inhibition Assays

A. Inhibition of Phosphorylation of EGF Receptor Peptide

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This assay was carried out in the presence of 10 mM MgCl₂, 25 mM β -glycerophosphate, 10% glycerol and 100 mM HEPES buffer at pH 7.6. For a typical IC₅₀ determination, a stock solution was prepared containing all of the above components and activated p38 (5 nM). The stock solution was aliquotted into vials. A fixed volume of DMSO or inhibitor in DMSO (final concentration of DMSO in reaction was 5%) was introduced to each vial, mixed and incubated for 15 minutes at room temperature. EGF receptor peptide, KRELVEPLTPSGEAPNQALLR, a phosphoryl acceptor in p38-catalyzed kinase reaction (1), was added to each vial to a final concentration of 200 μ M. The kinase reaction was initiated with ATP (100 μ M) and the vials were incubated at 30°C. After 30 minutes, the reactions were quenched with equal volume of 10% trifluoroacetic acid (TFA).

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The phosphorylated peptide was quantified by HPLC analysis. Separation of phosphorylated peptide from

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the unphosphorylated peptide was achieved on a reverse phase column (Deltapak, 5 μ m, C18 100D, Part no. 011795) with a binary gradient of water and acetonitrile, each containing 0.1% TFA. IC_{50} (concentration of inhibitor yielding 50% inhibition) was determined by plotting the percent (%) activity remaining against inhibitor concentration.

B. Inhibition of ATPase Activity

This assay is carried out in the presence of 10 mM $MgCl_2$, 25 mM β -glycerophosphate, 10% glycerol and 100 mM HEPES buffer at pH 7.6. For a typical K_i determination, the K_m for ATP in the ATPase activity of activated p38 reaction is determined in the absence of inhibitor and in the presence of two concentrations of inhibitor. A stock solution is prepared containing all of the above components and activated p38 (60 nM). The stock solution is aliquotted into vials. A fixed volume of DMSO or inhibitor in DMSO (final concentration of DMSO in reaction was 2.5%) is introduced to each vial, mixed and incubated for 15 minutes at room temperature. The reaction is initiated by adding various concentrations of ATP and then incubated at 30°C. After 30 minutes, the reactions are quenched with 50 μ l of EDTA (0.1 M, final concentration), pH 8.0. The product of p38 ATPase activity, ADP, is quantified by HPLC analysis.

Separation of ADP from ATP is achieved on a reversed phase column (Supelcosil, LC-18, 3 μ m, part no. 5-8985) using a binary solvent gradient of following composition: Solvent A - 0.1 M phosphate buffer containing 8 mM tetrabutylammonium hydrogen sulfate

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(Sigma Chemical Co., catalogue no. T-7158), Solvent B - Solvent A with 30% methanol.

Ki is determined from the rate data as a function of inhibitor and ATP concentrations.

5 p38 inhibitors of this invention will inhibit the ATPase activity of p38.

C. Inhibition of IL-1, TNF, IL-6 and IL-8
Production in LPS-Stimulated PBMCs

10

Inhibitors were serially diluted in DMSO from a 20 mM stock. At least 6 serial dilutions were prepared. Then 4x inhibitor stocks were prepared by adding 4 μ l of an inhibitor dilution to 1 ml of RPMI1640 medium/10% fetal bovine serum. The 4x inhibitor stocks contained inhibitor at concentrations of 80 μ M, 32 μ M, 12.8 μ M, 5.12 μ M, 2.048 μ M, 0.819 μ M, 0.328 μ M, 0.131 μ M, 0.052 μ M, 0.021 μ M etc. The 4x inhibitor stocks were pre-warmed at 37°C until use.

20

Fresh human blood buffy cells were separated from other cells in a Vacutainer CPT from Becton & Dickinson (containing 4 ml blood and enough DPBS without Mg^{2+}/Ca^{2+} to fill the tube) by centrifugation at 1500 x g for 15 min. Peripheral blood mononuclear cells (PBMCs), located on top of the gradient in the Vacutainer, were removed and washed twice with RPMI1640 medium/10% fetal bovine serum. PBMCs were collected by centrifugation at 500 x g for 10 min. The total cell number was determined using a Neubauer Cell Chamber and the cells were adjusted to a concentration of 4.8×10^6 cells/ml in cell culture medium (RPMI1640 supplemented with 10% fetal bovine serum).

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Alternatively, whole blood containing an anti-coagulant was used directly in the assay.

100 μ l of cell suspension or whole blood were placed in each well of a 96-well cell culture plate.

5 Then 50 μ l of the 4x inhibitor stock was added to the cells. Finally, 50 μ l of a lipopolysaccharide (LPS) working stock solution (16 ng/ml in cell culture medium) was added to give a final concentration of 4 ng/ml LPS in the assay. The total assay volume of the vehicle control
10 was also adjusted to 200 μ l by adding 50 μ l cell culture medium. The PBMC cells or whole blood were then incubated overnight (for 12-15 hours) at 37° C/5% CO₂ in a humidified atmosphere.

The next day the cells were mixed on a shaker
15 for 3-5 minutes before centrifugation at 500 x g for 5 minutes. Cell culture supernatants were harvested and analyzed by ELISA for levels of IL-1 β (R & D Systems, Quantikine kits, #DBL50), TNF- α (BioSource, #KHC3012), IL-6 (Endogen, #EH2-IL6) and IL-8 (Endogen, #EH2-IL8)
20 according to the instructions of the manufacturer. The ELISA data were used to generate dose-response curves from which IC₅₀ values were derived.

Results for the kinase assay ("kinase"; subsection A, above), IL-1, and TNF in LPS-stimulated
25 PBMC's ("cell") and IL-1, TNF, and IL-6 in whole blood ("WB") for various p38 inhibitors of this invention are shown in Table 7 below:

Table 7.

Compound	M.W.	Kinase IC50 (uM)	Cell IL-1 IC50 (uM)	Cell TNF IC50 (uM)	WB IL-1 IC50 (uM)	WB TNF IC50 (uM)	WB IL-6 IC50 (uM)
13	559.55	0.031	0.012	0.022	0.140	0.055	0.083
9	489.43	1.0	0.05	0.05	12.2	20.0	11.0
10	544.51	5.0			2.2	4.3	0.8

Other p38 inhibitors of this invention will also inhibit phosphorylation of EGF receptor peptide, and will inhibit the production of IL-1, TNF and IL-6, as well as IL-8, in LPS-stimulated PBMCs or in whole blood.

D. Inhibition of IL-6 and IL-8
Production in IL-1-Stimulated PBMCs

This assay is carried out on PBMCs exactly the same as above except that 50 μ l of an IL-1b working stock solution (2 ng/ml in cell culture medium) is added to the assay instead of the (LPS) working stock solution.

Cell culture supernatants are harvested as described above and analyzed by ELISA for levels of IL-6 (Endogen, #EH2-IL6) and IL-8 (Endogen, #EH2-IL8) according to the instructions of the manufacturer. The ELISA data are used to generate dose-response curves from which IC50 values were derived.

E. Inhibition of LPS-Induced
Prostaglandin Endoperoxide Synthase-2
(PGHS-2, or COX-2) Induction in PBMCs

Human peripheral mononuclear cells (PBMCs) are
5 isolated from fresh human blood buffy coats by
centrifugation in a Vacutainer CPT (Becton & Dickinson).
15 x 10⁶ cells are seeded in a 6-well tissue culture dish
containing RPMI 1640 supplemented with 10% fetal bovine
serum, 50U/ml penicillin, 50 µg/ml streptomycin, and 2 mM
10 L-glutamine. Compounds are added at 0.2, 2.0 and 20 µM
final concentrations in DMSO. LPS is then added at a
final concentration of 4 ng/ml to induce enzyme
expression. The final culture volume is 10 ml/well.

After overnight incubation at 37°C, 5% CO₂, the
15 cells are harvested by scraping and subsequent
centrifugation, the supernatant is removed, and the cells
are washed twice in ice-cold DPBS (Dulbecco's phosphate
buffered saline, BioWhittaker). The cells are lysed on
ice for 10 min in 50 µl cold lysis buffer (20 mM Tris-
20 HCl, pH 7.2, 150 mM NaCl, 1% Triton-X-100, 1% deoxycholic
acid, 0.1% SDS, 1 mM EDTA, 2% aprotinin (Sigma), 10 µg/ml
pepstatin, 10 µg/ml leupeptin, 2 mM PMSF, 1 mM
benzamidine, 1 mM DTT) containing 1 µl Benzonase (DNase
from Merck). The protein concentration of each sample is
25 determined using the BCA assay (Pierce) and bovine serum
albumin as a standard. Then the protein concentration of
each sample is adjusted to 1 mg/ml with cold lysis
buffer. To 100 µl lysate an equal volume of 2xSDS PAGE
loading buffer is added and the sample is boiled for 5
30 min. Proteins (30 µg/lane) are size-fractionated on 4-
20% SDS PAGE gradient gels (Novex) and subsequently
transferred onto nitrocellulose membrane by

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electrophoretic means for 2 hours at 100 mA in Towbin transfer buffer (25 mM Tris, 192 mM glycine) containing 20% methanol. After transfer, the membrane is pretreated for 1 hour at room temperature with blocking buffer (5% non-fat dry milk in DPBS supplemented with 0.1% Tween-20) and washed 3 times in DPBS/0.1% Tween-20. The membrane is incubated overnight at 4°C with a 1: 250 dilution of monoclonal anti-COX-2 antibody (Transduction Laboratories) in blocking buffer. After 3 washes in DPBS/0.1% Tween-20, the membrane is incubated with a 1:1000 dilution of horseradish peroxidase-conjugated sheep antiserum to mouse Ig (Amersham) in blocking buffer for 1 h at room temperature. Then the membrane is washed again 3 times in DPBS/0.1% Tween-20. An ECL detection system (SuperSignal™ CL-HRP Substrate System, Pierce) is used to determine the levels of expression of COX-2.

EXAMPLE 8

ZAP70 Inhibition Assay

The activity of ZAP 70 is measured by determining the phosphorylation poly E4Y (Sigma Chemicals, St Louis MO) with γ -³³P ATP (NEN, Boston, MA). Reactions are carried out at room temperature in a buffer containing 100 mM HEPES, pH 7.5, 10 mM MgCl₂, 25 mM NaCl, 1 mM DTT and 0.01% BSA. Final concentrations of ZAP70 and poly E4Y are 20 nM and 5 μ M respectively. Test compounds in DMSO (final concentration of compounds was 30 μ M in 1.5% DMSO) are added to the reaction mixture containing the above-described components. The reaction is initiated by addition of γ -³³P ATP (final concentration 20 μ M, specific activity = 0.018 Ci/mmol). The reaction is allowed to proceed for 12 minutes and then is quenched

by the addition of 10% TCA containing 200 mM ATP. The quenched reaction is harvested onto GF/C glass fiber filter plates (Packard, Meriden, CT) using a Tomtec 9600 cell harvester (Tomtec, Hamden, CT). The plates are
5 washed with 5% TCA containing 1 mM ATP and water. 50 μ l of scintillation fluid is added to the plates, which are then counted using a Packard scintillation counter (Packard, Meriden, CT). IC50 values for inhibitory
10 compounds were determined using the same assay at a series of compound concentrations.

While we have hereinbefore presented a number of embodiments of this invention, it is apparent that our basic construction can be altered to provide other
15 embodiments which utilize the methods of this invention.

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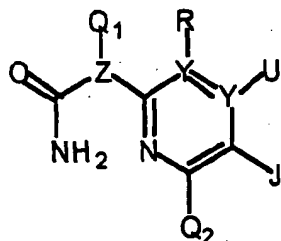
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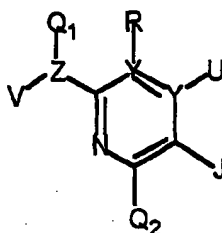
CLAIMS

We claim:

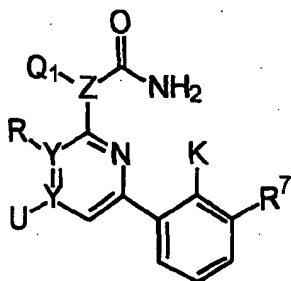
1. A compound of the formula:



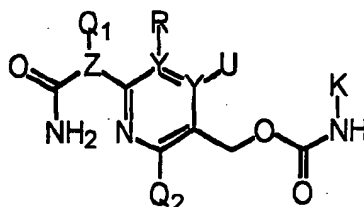
(Ia),



(Ib),



(Ic)



or (Id),

wherein each of Q_1 and Q_2 are independently selected from a phenyl or 5-6 membered aromatic heterocyclic ring system; or a 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring;

wherein the rings that make up Q_1 are substituted with 1 to 4 substituents, each of which is independently selected from halo; C_1 - C_3 alkyl optionally substituted with NR'_2 , OR' , CO_2R' or $CONR'_2$; O -(C_1 - C_3)-alkyl optionally substituted with NR'_2 , OR' , CO_2R' or

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CONR'₂; NR'₂; OCF₃; CF₃; NO₂; CO₂R'; CONR'; SR';
 S(O₂)N(R')₂; SCF₃; CN; N(R')C(O)R⁴; N(R')C(O)OR⁴;
 N(R')C(O)C(O)R⁴; N(R')S(O₂)R⁴; N(R')R⁴; N(R⁴)₂; OR⁴;
 OC(O)R⁴; OP(O)₃H₂; or N=C-N(R')₂;

wherein the rings that make up Q₂ are optionally substituted with up to 4 substituents, each of which is independently selected from halogen; C₁-C₃ straight or branched alkyl optionally substituted with R', NR'₂, OR', CO₂R', S(O₂)N(R')₂, N=C-N(R')₂, R³, O-P(O₃)H₂, or CONR'₂; O-(C₁-C₃)-alkyl; O-(C₁-C₃)-alkyl optionally substituted with NR'₂, OR', CO₂R', S(O₂)N(R')₂, N=CR'-N(R')₂, R³, OP(O₃)H₂, or CONR'₂; NR'₂; OCF₃; CF₃; NO₂; CO₂R'; CONR'₂; R³; OR³; NR³₂; SR³; C(O)R³; C(O)N(R')R³; C(O)OR³; SR'; S(O₂)N(R')₂; SCF₃; N=CR'-N(R')₂; OR⁴; O-CO₂R⁴; N(R')C(O)R⁴; N(R')C(O)OR⁴; N(R')C(O)C(O)R⁴; N(R')S(O₂)R⁴; N(R')R⁴; N(R⁴)₂; OR⁴; OC(O)R⁴; OP(O)₃H₂; K; or CN;

wherein each R' is independently selected from hydrogen; (C₁-C₃)-alkyl; (C₂-C₃)-alkenyl or alkynyl; phenyl or phenyl substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl; or a 5-6 membered heterocyclic ring system optionally substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl;

wherein each R is independently selected from hydrogen, -R², -N(R²)₂, -OR², SR², -C(O)-N(R²)₂, -S(O₂)-N(R²)₂, -C(O)-OR² or -C(O)R² wherein two adjacent R are optionally bound to one another and, together with each Y to which they are respectively bound, form a 4-8 membered carbocyclic or heterocyclic ring;

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wherein each R^2 is independently selected from hydrogen; or (C₁-C₃)-alkyl or (C₁-C₃)-alkenyl, each optionally substituted with -N(R')₂, -OR', SR', -O-C(O)-N(R')₂, -C(O)-N(R')₂, -S(O₂)-N(R')₂, -C(O)-OR', -NSO₂R⁴, -NSO₂R³, -C(O)N(R')(R³), -NC(O)R⁴, -N(R')(R³), -N(R')(R⁴), -C(O)R³, -C(O)N(R')(R⁴), -N(R⁴)₂, -C(O)N=C(NH)₂ or R³;

wherein each R^3 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or heterocyclic ring systems each optionally substituted with R', R⁴, -C(O)R', -C(O)R⁴, -C(O)OR⁴ or -K; or an 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R', R⁴, -C(O)R', -C(O)R⁴, -C(O)OR⁴ or -K;

wherein each R^4 is independently selected from R'; (C₁-C₇)-straight or branched alkyl optionally substituted with R', N(R')₂, OR', CO₂R', CON(R')₂, SO₂N(R')₂ or SO₂N(R⁵)₂; or a 5-6 membered carbocyclic or heterocyclic ring system optionally substituted with N(R')₂, OR', CO₂R', CON(R')₂, SO₂N(R')₂ or SO₂N(R⁵)₂;

wherein each R^5 is independently selected from hydrogen, (C₁-C₃)-alkyl, or (C₁-C₃)-alkenyl; each optionally substituted with -N(R')₂, -OR', SR', -C(O)-N(R')₂, -S(O₂)-N(R')₂, -C(O)-OR', -N-S(O₂)(R'), -NSO₂R⁶, -C(O)N(R')(R⁶), -NC(O)R', -N(R')(R⁶), -C(O)R⁶, -C(O)N=C(NH)₂ or R⁶;

wherein each R^6 is independently selected from 5-8 membered aromatic or non-aromatic carbocyclic or

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heterocyclic ring systems each optionally substituted with R' , $-C(O)R'$ or $-C(O)OR'$; or an 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring each optionally substituted with R' , $-C(O)R'$ or $C(O)OR'$;

wherein R^7 is selected from H, halogen, or a (C_1-C_3) straight chain or branched alkyl;

wherein each Y is independently selected from N or C. If either Y is N, then R or U attached to Y is a lone pair of electrons;

wherein Z is CH, N, $C(OCH_3)$, $C(CH_3)$, $C(NH_2)$, $C(OH)$ or $C(F)$;

wherein each U is independently selected from R or J;

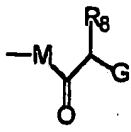
wherein each J is independently selected from a (C_1-C_4) straight chain or branched alkyl derivative substituted with T;

wherein each T is independently selected from either $O(V)$ or $N(H)(V)$;

wherein each V is independently selected from $C(O)N=C(R)(N(R)_2)$, wherein the two geminal R on the nitrogen are optionally bound to one another to form a 4-8 membered carbocyclic or heterocyclic ring;

wherein each K is independently selected from a (C_1-C_4) straight chain or branched alkyl derivative substituted with D, or $-OP(O)(OH)_2$;

wherein each D is independently selected from

either enantiomer of  ;

wherein each M is independently selected from either O or NH;

wherein each G is independently selected from NH₂, OH, or H;

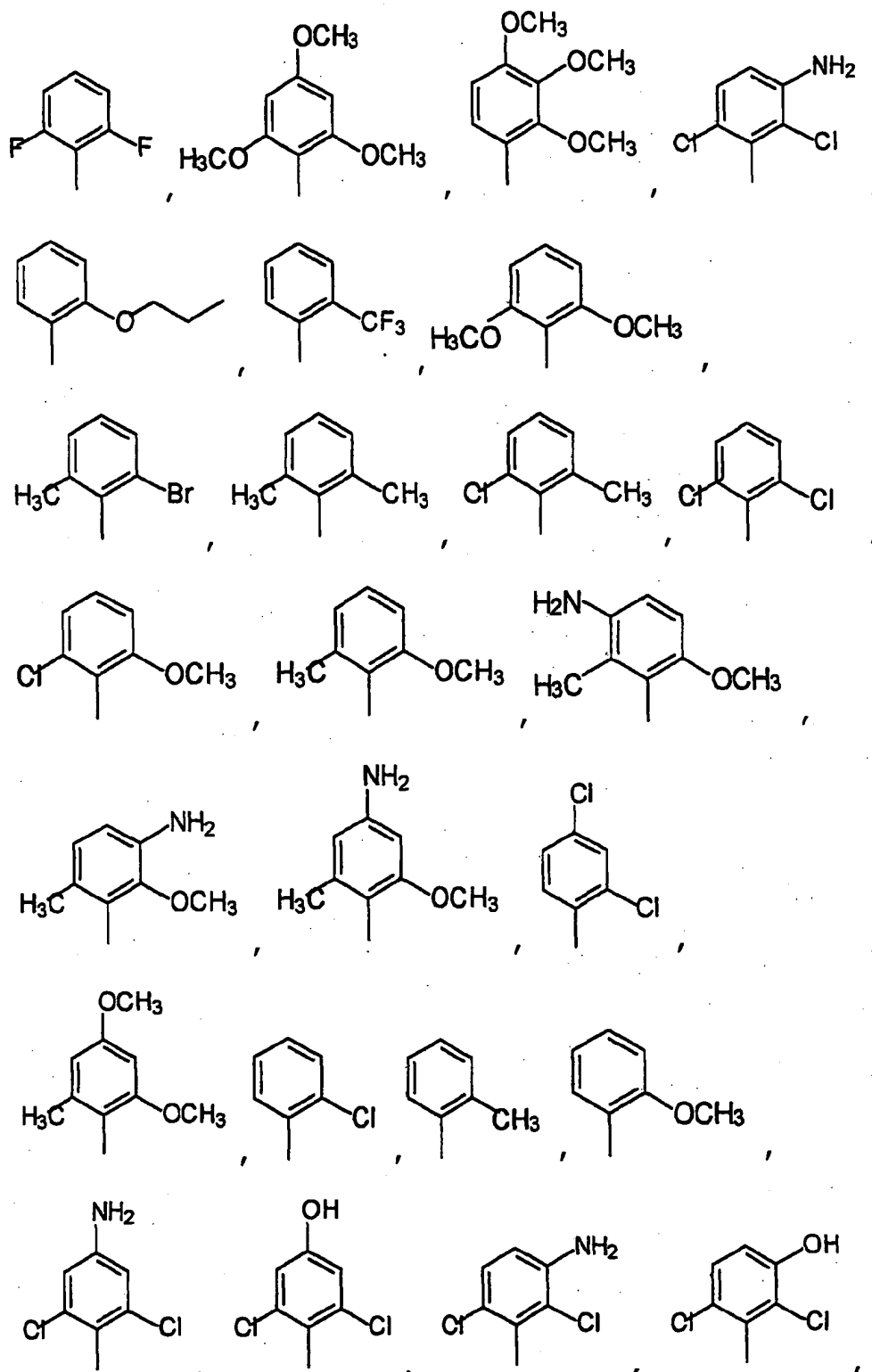
wherein each R₈ is independently selected from H, OH, C(O)OH, (C₁-C₇)-straight or branched alkyl optionally substituted with N(R')₂, OR', CO₂R', CON(R')₂, or SO₂N(R')₂; or a 5-6 membered carbocyclic, heterocyclic or heteroaryl ring system optionally substituted with N(R')₂, OR', CO₂R', CON(R')₂, or SO₂N(R')₂; wherein G and R₈ are optionally bound to one another to form a ring.

2. The compound according to claim 1, wherein Q₁ is selected from phenyl or pyridyl containing 1 to 3 substituents independently selected from chloro, fluoro, bromo, -CH₃, -OCH₃, -OH, -CF₃, -OCF₃, -O(CH₂)₂CH₃, NH₂, 3,4-methylenedioxy, -N(CH₃)₂, -NH-S(O)₂-phenyl, -NH-C(O)O-CH₂-4-pyridine, -NH-C(O)CH₂-morpholine, -NH-C(O)CH₂-N(CH₃)₂, -NH-C(O)CH₂-piperazine, -NH-C(O)CH₂-pyrrolidine, -NH-C(O)C(O)-morpholine, -NH-C(O)C(O)-piperazine, -NH-C(O)C(O)-pyrrolidine, -O-C(O)CH₂-N(CH₃)₂, or -O-(CH₂)₂-N(CH₃)₂ and wherein at least one of said substituents is in the ortho position.

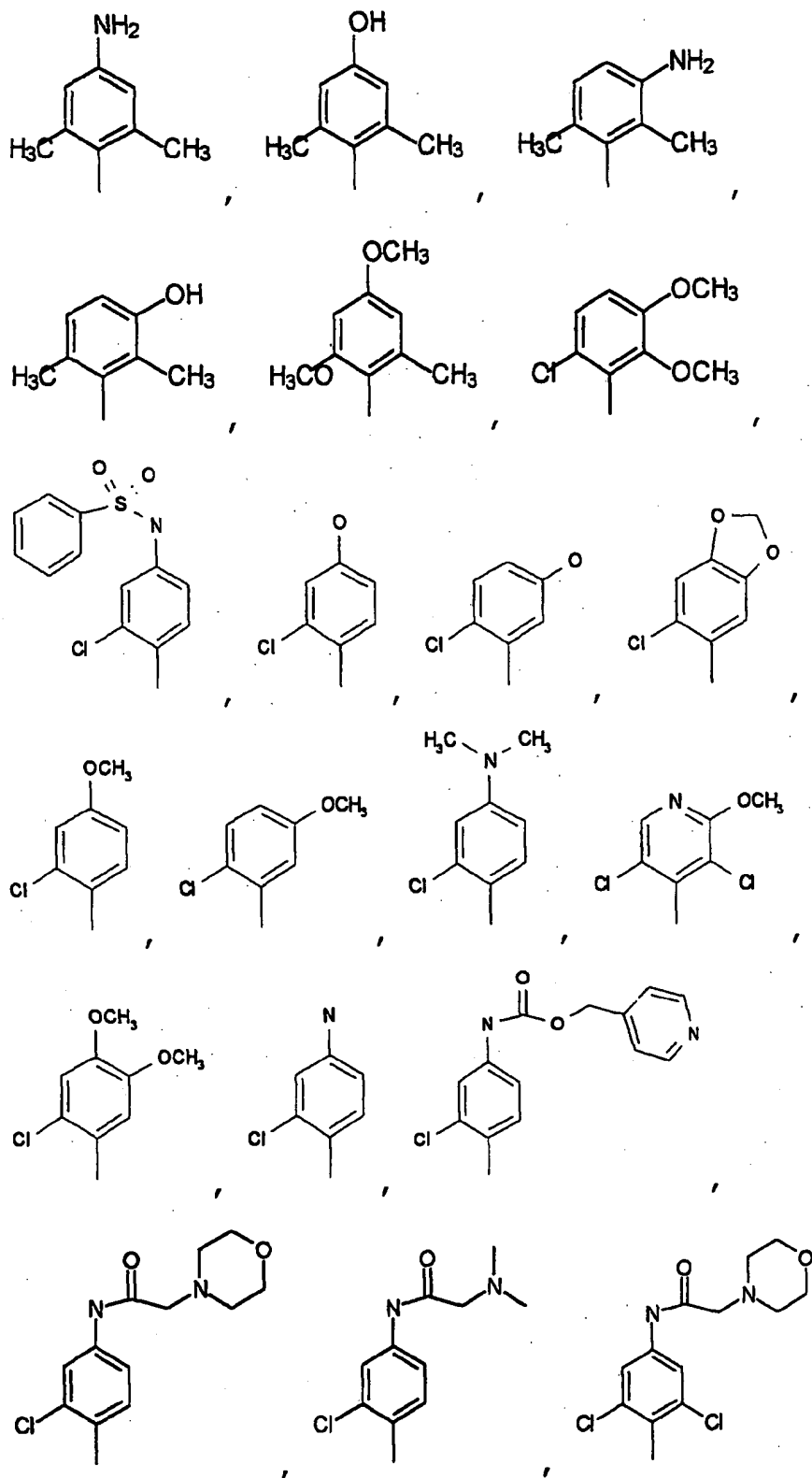
3. The compound according to claim 2, wherein Q₁ contains at least two substituents, both of which are in the ortho position.

4. The compound according to claim 2, wherein Q₁ is selected from:

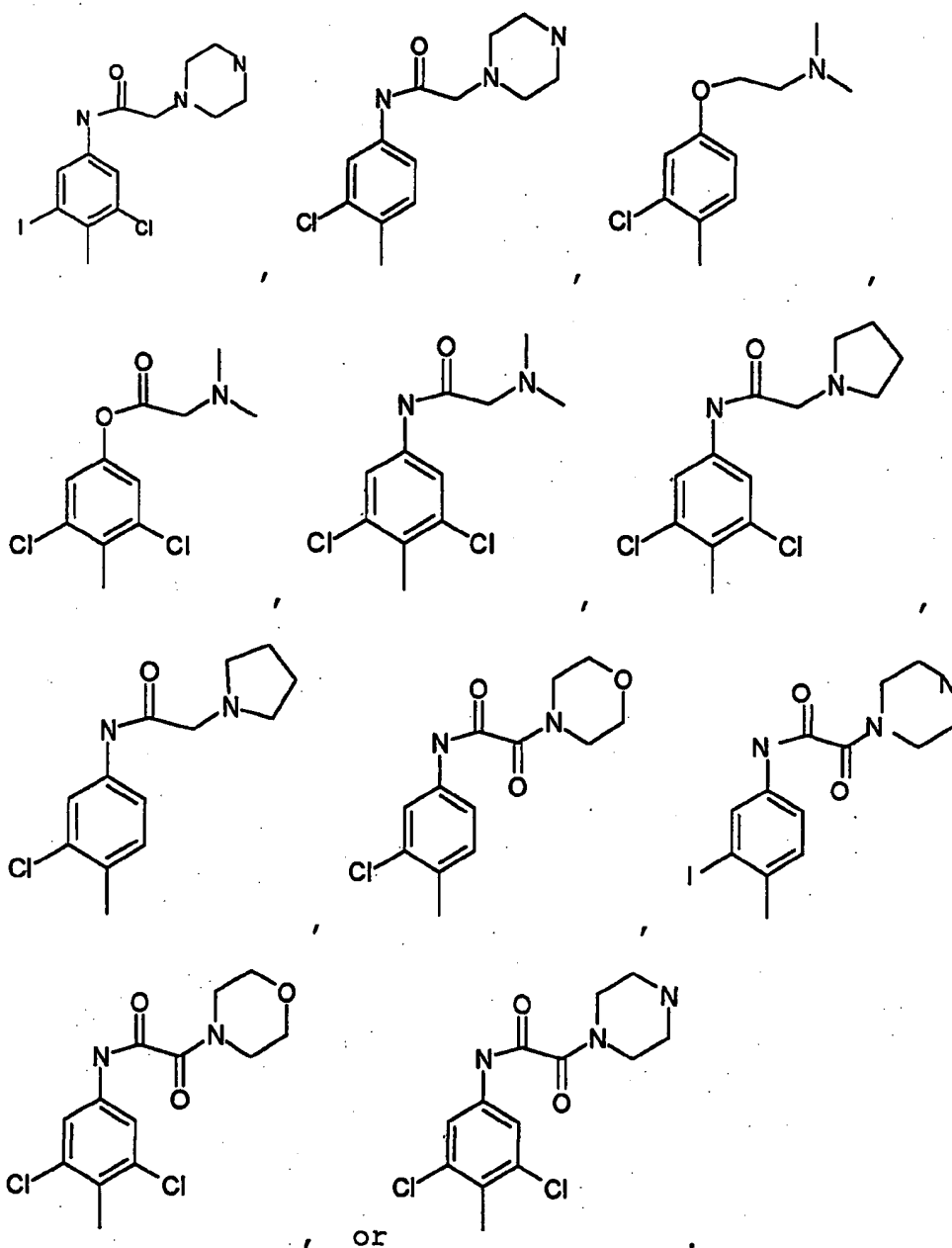
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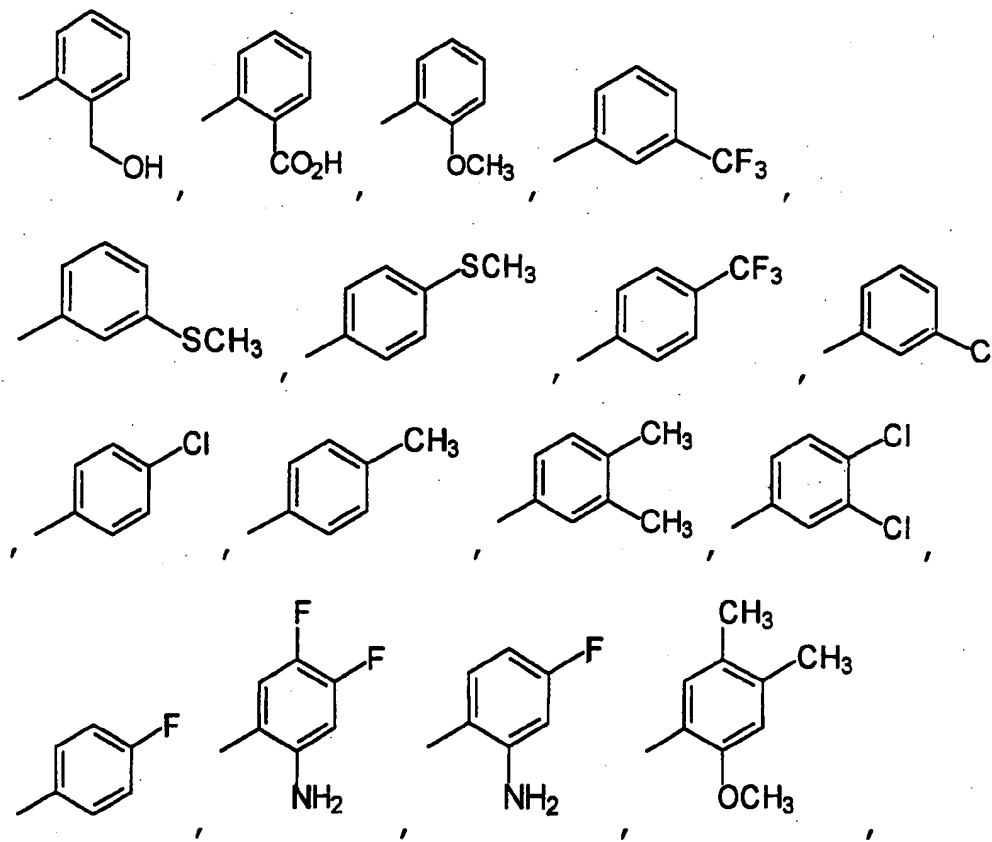
5. The compound according to claim 4, wherein Q_1 is selected from 2-fluoro-6-trifluoromethylphenyl, 2,6-difluorophenyl, 2,6-dichlorophenyl, 2-chloro-4-hydroxyphenyl, 2-chloro-4-aminophenyl, 2,6-dichloro-4-aminophenyl, 2,6-dichloro-3-aminophenyl, 2,6-dimethyl-4-hydroxyphenyl, 2-methoxy-3,5-dichloro-4-pyridyl, 2-

-71-

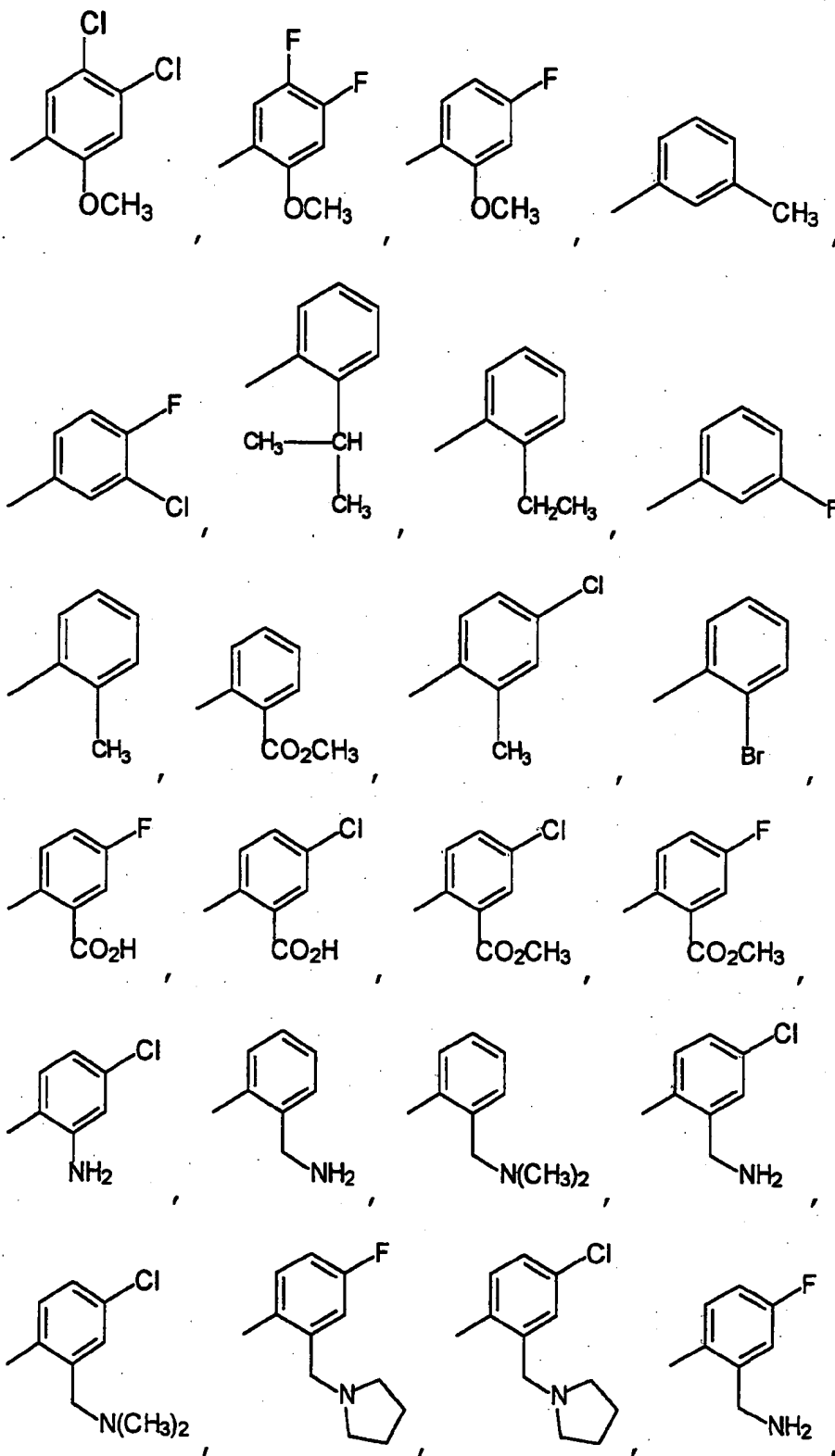
chloro-4,5 methylenedioxy phenyl, or 2-chloro-4-(N-2-morpholino-acetamido)phenyl.

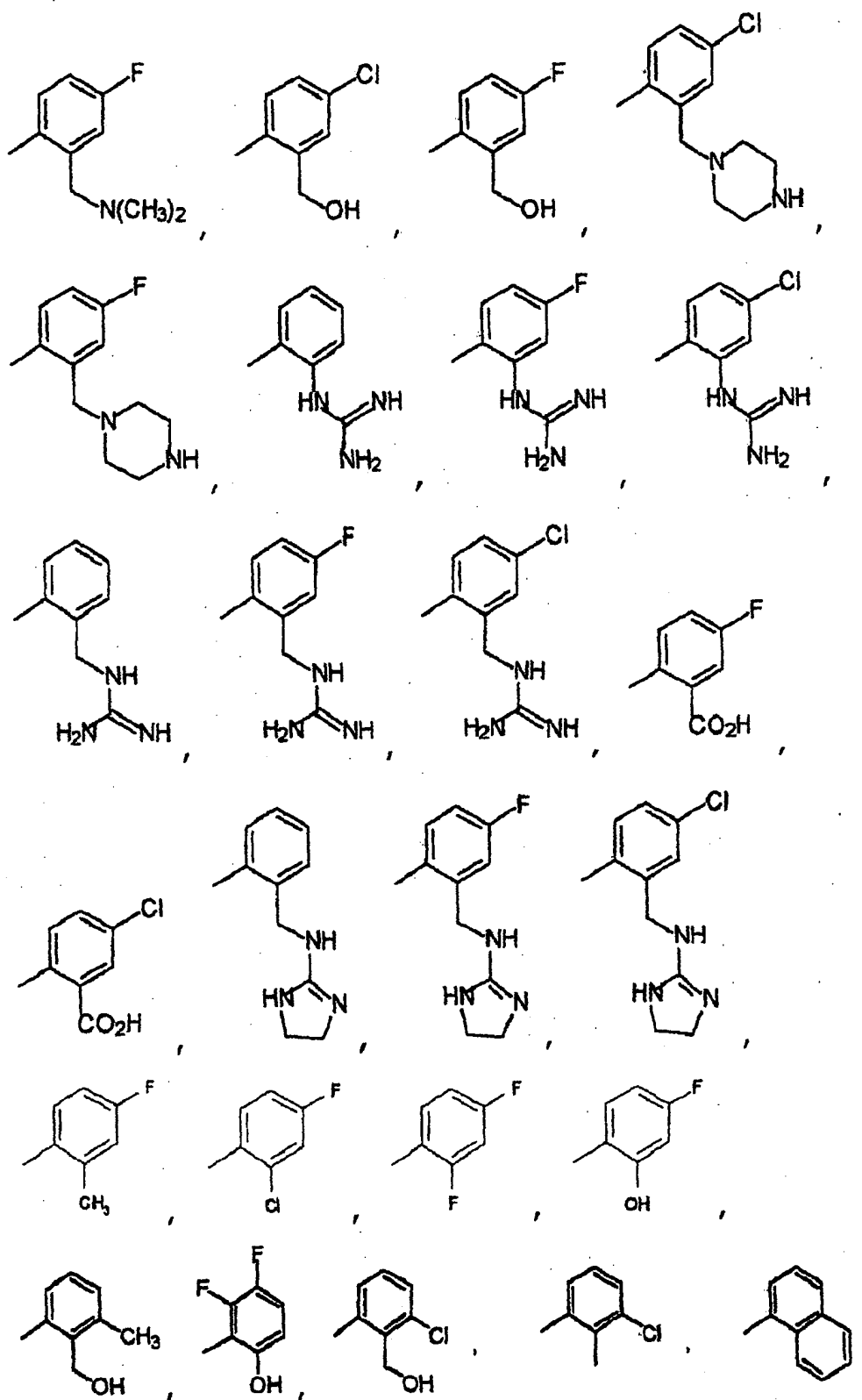
6. The compound according to claim 1, wherein Q_2 is selected from phenyl, pyridyl or naphthyl and wherein Q_2 optionally contains up to 3 substituents, each of which is independently selected from chloro, fluoro, bromo, methyl, ethyl, isopropyl, $-OCH_3$, $-OH$, $-NH_2$, $-CF_3$, $-OCF_3$, $-SCH_3$, $-OCH_3$, $-C(O)OH$, $-C(O)OCH_3$, $-CH_2NH_2$, $-N(CH_3)_2$, $-CH_2$ -pyrrolidine and $-CH_2OH$.

7. The compound according to claim 6, wherein Q_2 is selected from:



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unsubstituted 2-pyridyl or unsubstituted phenyl.

8. The compound according to claim 7, where Q₂ is selected from phenyl, 2-isopropylphenyl, 3,4-dimethylphenyl, 2-ethylphenyl, 3-fluorophenyl, 2-methylphenyl, 3-chloro-4-fluorophenyl, 3-chlorophenyl, 2-carbomethoxyphenyl, 2-carboxyphenyl, 2-methyl-4-chlorophenyl, 2-bromophenyl, 2-pyridyl, 2-methylenedihydroxyphenyl, 4-fluorophenyl, 2-methyl-4-fluorophenyl, 2-chloro-4-fluorophenyl, 2,4-difluorophenyl, 2-hydroxy-4-fluorophenyl or 2-methylenedihydroxy-4-fluorophenyl, 1-naphthyl, 3-chloro-2-methylenedihydroxy, 3-chloro-2-methyl, or 4-fluoro-2-methyl.

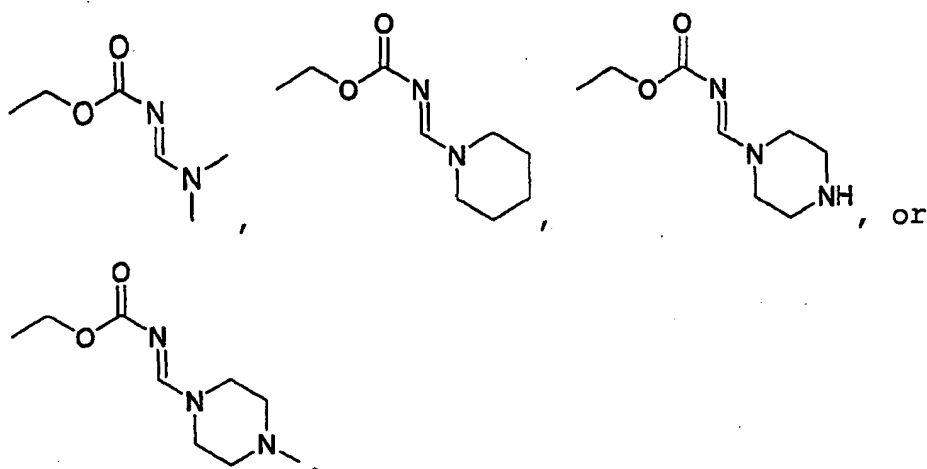
9. The compound according to claim 1, wherein each Y is C.

10. The compound according to claim 9, wherein the R attached to Y is independently selected from hydrogen or methyl.

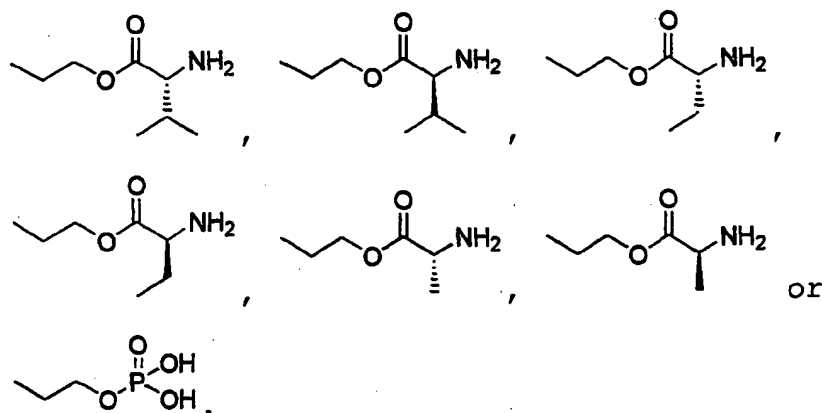
11. The compound according to claim 1, wherein J is a 0-8 atom chain terminating in an alcohol, amine, carboxylic acid, ester, amide, amidine or heterocycle.

12. The compound according to claim 11, wherein J is selected from:

-75-



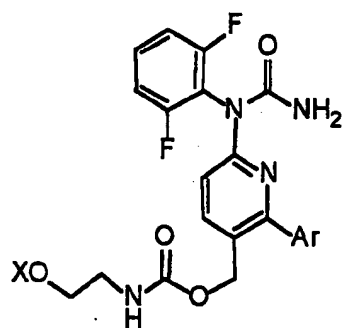
13. The compound according to claim 1 wherein K is selected from:



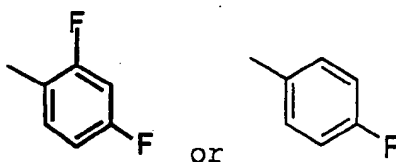
14. The compound according to claim 1, wherein the compound is selected from any one of the compounds depicted in Tables 1-3.

15. The compound according to claim 1, wherein the compound is

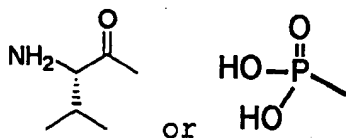
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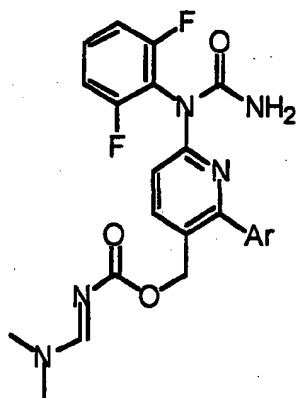
wherein Ar is



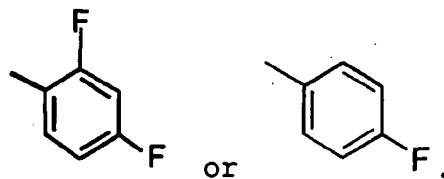
and X =



16. The compound according to claim 1, wherein the compound is

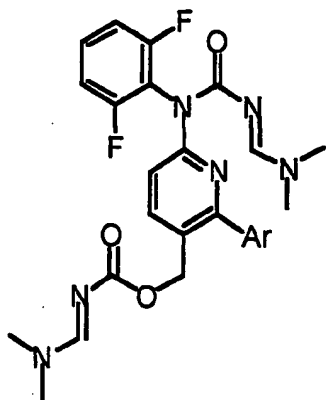


wherein Ar is

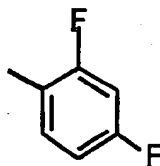


-77-

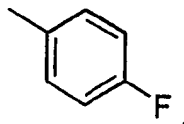
17. The compound according to claim 1, wherein the compound is



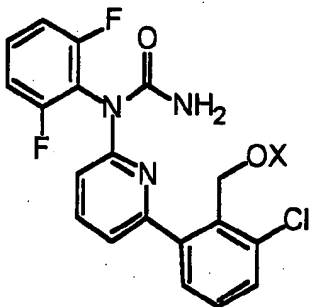
wherein Ar is



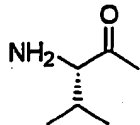
or



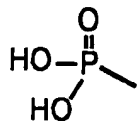
18. The compound according to claim 1, wherein the compound is



wherein X =

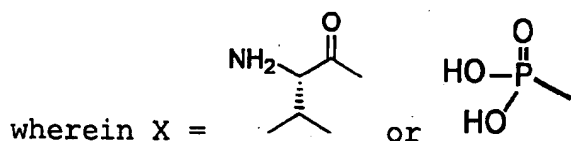
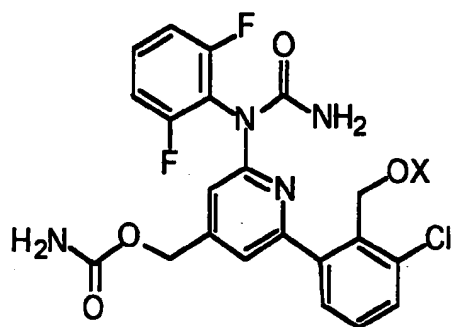


or



19. The compound according to claim 1, wherein the compound is

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20. A pharmaceutical composition comprising an amount of a compound according to any one of claims 1 to 19 effective to inhibit p38, and a pharmaceutically acceptable carrier.

21. A method of treating or preventing inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, neurodegenerative diseases, allergies, reperfusion/ischemia in stroke, heart attacks, angiogenic disorders, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, thrombin-induced platelet aggregation or conditions associated with prostaglandin endoperoxidase synthase-2 in a patient, said method comprising administering to said patient a composition according to claim 20.

22. The method according to claim 21, wherein said method is used to treat or prevent an inflammatory disease selected from acute pancreatitis, chronic

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pancreatitis, asthma, allergies, or adult respiratory distress syndrome.

23. The method according to claim 21, wherein said method is used to treat or prevent an autoimmune disease selected from glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, atopic dermatitis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, psoriasis, or graft vs. host disease.

~~24. The method according to claim 21, wherein~~
said method is used to treat or prevent a destructive bone disorders selected from osteoarthritis, osteoporosis or multiple myeloma-related bone disorder.

25. The method according to claim 21, wherein said method is used to treat or prevent a proliferative disease selected from acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, or multiple myeloma.

26. The method according to claim 21, wherein said method is used to treat or prevent an infectious disease selected from sepsis, septic shock, or Shigellosis.

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27. The method according to claim 21, wherein said method is used to treat or prevent a viral disease selected from acute hepatitis infection, HIV infection or CMV retinitis.

28. The method according to claim 21, wherein said method is used to treat or prevent a neurodegenerative disease selected from Alzheimer's disease, Parkinson's disease, cerebral ischemia or neurodegenerative disease caused by traumatic injury.

29. The method according to claim 21, wherein said method is used to treat or prevent ischemia/reperfusion in stroke or myocardial ischemia, renal ischemia, heart attacks, organ-hypoxia or thrombin-induced platelet aggregation.

30. The method according to claim 21, wherein said method is used to treat or prevent a condition associated with prostaglandin endoperoxide synthase-2 selected from edema, fever, analgesia or pain.

31. The method according to claim 30, wherein said pain is selected from neuromuscular pain, headache, cancer pain, dental pain or arthritis pain.

32. The method according to claim 21, wherein said method is used to treat or prevent an angiogenic disorder selected from solid tumors, ocular neovascularization, or infantile haemangiomas.

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33. A method of treating or preventing a p38-mediated disease, said method comprising administering to said patient a composition according to claim 20.

34. A pharmaceutical composition comprising an amount of a compound according to any one of claims 1 to 19 effective to inhibit ZAP70, and a pharmaceutically acceptable carrier.

35. A method of treating or preventing organ or tissue rejection associated with transplantation, autoimmune disease, cancer, multiple sclerosis, graft versus host disease, and Kawasaki syndrome, said method comprising administering to said patient a composition according to claim 34.

36. The method according to claim 35, wherein said method is used to treat or prevent an autoimmune disease selected from rheumatoid arthritis, systemic lupus erythematosus (SLE), psoriasis, Sjogren's Syndrome, thyroiditis, pulmonary fibrosis, bronchiolitis obliterans, hemolytic anemia and Wegener's granulomatosis.

37. The method according to claim 35, wherein said method is used to treat or prevent a cancer selected from leukemia and lymphoma.

38. A method of treating or preventing a ZAP70-mediated disease, said method comprising administering to said patient a composition according to claim 34.

INTERNATIONAL SEARCH REPORT

Internat. Application No.

PCT/US 01/25015

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D213/75 C07D401/12 A61K31/4418 A61P7/00 A61P9/00
A61P17/00 A61P25/00 A61P31/00 C07F9/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 58502 A (BELLON STEVEN ;BEMIS GUY (US); VERTEX PHARMA (US); COCHRAN JOHN (U) 18 November 1999 (1999-11-18) cited in the application page 71, line 17 - line 20; claims; examples	1-40
E	WO 01 70695 A (EVINDAR GHOTAS ;BEMIS GUY (US); VERTEX PHARMA (US); SALITURO FRANC) 27 September 2001 (2001-09-27) claims 1,8,11-23	1,13,14, 18,20-40

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

22 January 2002

Date of mailing of the international search report

04/02/2002

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FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Claims Nos.: 1-13, 20-40 (partially)

Present claims 1-13 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the examples and to the compounds according to claims 1-13 of the present formulae (Ia), (Ib), (Ic), (Id) in which Q1 has the meanings as defined in claim 4, and Q2 has the meanings as defined in claim 7, and also to the use of these compounds according to claims 20-40.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

relation on patent family members

Intern: Application No
PCT/US 01/25015

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9958502	A	18-11-1999	AU 3792399 A	29-11-1999
			BG 105031 A	31-10-2001
			BR 9911786 A	03-04-2001
			CN 1306512 T	01-08-2001
			EP 1077943 A1	28-02-2001
			NO 20005673 A	10-01-2001
			PL 344046 A1	24-09-2001
			SK 16882000 A3	10-05-2001
			WO 9958502 A1	18-11-1999
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